

Final Report
Evaluation of Biomass for Uranium
Recovery from Process Streams

by
SENES Consultants Limited
In Association with the
Institute for Biotechnology Research

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25 September 1985

Energy Mines and Resources Canada
Canada Centre for Mineral and Energy Technology
555 Booth Street, Room 304
Ottawa, Ontario

Attention: Dr. V. Sanmugasunderam
Research Scientist, Biotechnology Section

Dear Sir:

Re: Evaluation of Biomass for Uranium Recovery from from Process
Streams D.S.S. No. 26SQ.23440-4-9173

It is with great pleasure we submit 10 copies of our final reports for Stage I, II and III. This program has confirmed the viability of biomass for uranium recovery and has identified in mixed culture, Streptomyces and Rhizopus organisms as the most suited for practical applications. As expected, recoveries for these organisms are somewhat lower than those quoted in the literature when actual process liquid rather than pure solutions are employed.

We greatly appreciate the opportunity of working on this project and remain,

Yours very truly,

SENE CONSULTANTS LIMITED

R.A. Knapp, P.Eng.

J.M. Scharer, Ph.D.

sd

EXECUTIVE SUMMARY

This study on the utilization of biomass for uranium recovery from process streams was conducted in three stages. The Stage I study reviewed the literature, selected five biomasses for investigation, and outlined the basis for the experimental testwork. Stage II was the laboratory testing program and Stage III was a technical and economic evaluation of the biomasses tested.

The five biomasses tested were Rhizopus arrhizus, Streptomyces levoris, a mixed culture sewage sludge, Saccharomyces cerevisiae and Chlorella vulgaris.

The Stage II laboratory program determined growth rates and yields, factors affecting adsorption capacity, evaluated desorbing agents and assessed sorption mechanisms. The major results of the Stage II studies are summarized below.

- . with the exception of Chlorella vulgaris all biomasses displayed high growth yields. Rhizopus arrhizus was most readily harvested.
- . all biomasses displayed good adsorption characteristics. Factors such as low pH, elevated sulphate content and competing metals reduce adsorption capacity. Conversely thermal inactivation, and increased temperatures increased bioadsorption capacity.
- . uranium is readily desorbed with sodium bicarbonate. From the results it would appear that up to 10 cycles of adsorption/desorption could be effectively employed. Other desorbing agents including weak sulphuric acid, sodium EDTA and ammonium carbonate were ineffective.
- . simple sorption isotherms such as the Freundlich and Langmuir isotherms describe the data adequately.

The results of these Stage II laboratory studies, provided the basic design data for the Stage III evaluation of biomasses. The Stage III findings are summarized below.

- . biomasses are expensive to cultivate. Costs will typically be in the range of \$3 to \$5/kg.
- . conventional solids contact/separation equipment have been used for many biomass applications and are well suited to a uranium recovery process. Complete mix reactors for adsorption/desorption with thickeners and centrifuges for solid-liquid separation would appear to be most suited.
- . the major hurdle for full scale application is currently economics. Minor technical hurdles exist but are unlikely to be significant. The estimated cost of uranium recovery from the process mine water tested was in the range of \$30 to \$35/kg of U_3O_8 recovered.

The major conclusions of this work are as outlined below

- . Rhizopus arrhizus and the mixed culture sewage sludge were the best overall organisms tested. Chlorella vulgaris has little potential for application.
- . none of the organisms tested was selective to uranium. In this regard both cations and anions interfere with adsorption capacity.
- . process solutions may have characteristics such as low pH which may require pretreatment before bioadsorption.
- . bioadsorption capacities are significantly lower using process solutions than those reported for synthetic uranyl nitrate or chloride solutions.
- . sufficient basic data are now available to design a small pilot scale plant demonstration facility.

The major recommendations of this work are as follows.

- . a comparative assessment of biomass recovery processes versus alternative conventional or non-conventional technologies.
- . further basic studies concerning the biopolymers involved in bioadsorption and the identification of structural groups responsible for uranium complexing.
- . further study on the development of inexpensive growth media for biomass production.
- . further research into the development of robust immobilized biomass which retain high adsorption capacity.
- . in conjunction with pilot testing a standardized procedure for assessing and reporting bioadsorption capacity.

RÉSUMÉ ADMINISTRATIF

Cette étude portant sur l'utilisation de la biomasse en vue de la récupération de l'uranium des courants de procédés a été menée en trois étapes. La première étape consistait à passer en revue la littérature, à choisir cinq biomasses à étudier de même qu'à jeter les bases du travail expérimental en tant que tel. La deuxième étape consistait en un programme d'essais en laboratoire et la troisième était une évaluation technique et économique des biomasses à l'essai.

Les cinq biomasses à l'essai étaient : rhizopus arrhizus, streptomyces levoris, une culture mélangée de boue d'égouts, saccharomyces cerevisiae et chlorella vulgaris.

La deuxième étape a servi à déterminer les taux de croissance et les rendements de même que les facteurs modifiant la capacité d'absorption, à évaluer les agents de désorption et à déterminer les mécanismes d'absorption. Les principaux résultats de la deuxième étape de l'étude sont résumés ci-dessous :

- à l'exception de chlorella vulgaris, toutes les biomasses ont démontré un haut taux de croissance. Rhizopus arrhizus est celle dont le développement était le plus senti.
- toutes les biomasses ont démontré de bonnes caractéristiques d'absorption. Des facteurs tel qu'un pH faible, un contenu élevé en sulfate et des métaux en concurrence réduisent la capacité d'absorption. À l'inverse, l'inactivation thermique et l'augmentation des températures améliorent la capacité de bioabsorption.
- l'uranium se résorbe facilement avec du bicarbonate de sodium. On peut déduire des résultats que jusqu'à dix cycles d'absorption/désorption pourraient être employés facilement. D'autres agents de désorption incluant l'acide sulfurique de faible concentration, l'éthylènediaminetétracétique de sodium et le carbonate d'ammonium neutre sont demeurés sans résultat.
- de simples équations isothermiques telles que celles de Freundlich et Langmuir décrivent adéquatement les données.

Les résultats de la deuxième étape des études en laboratoire ont fourni les données de conception de base pour la troisième étape : l'évaluation des biomasses. Les résultats de la troisième étape sont résumés ci-dessous :

- . la culture des biomasses est dispendieuse. Les coûts varient normalement de 3 à 5 \$ le kilo.
- . l'équipement conventionnel de séparation et de contact des solides a été utilisé pour les biomasses et convient bien au procédé de récupération de l'uranium. Il semble que l'utilisation des réacteurs à mélange complet lors des cycles d'absorption/désorption avec épaississants et des réacteurs centrifuges pour la séparation des liquides et des solides soit toute indiquée.
- . l'obstacle majeur empêchant l'utilisation à plus grande échelle est présentement d'ordre économique. Il existe bien des obstacles mineurs d'ordre technique, mais ceux-ci sont minimes. Le coût de récupération de l'uranium à partir de l'eau de mines traitée à l'essai variait entre 30 à 35 \$ le kilo d' U_3O_8 récupéré, selon les estimations.

Les conclusions principales de cette étude sont les suivantes :

- . rhizopus arrhizus et la culture mélangée de boue d'égouts se sont avérés, parmi les organismes à l'essai et dans l'ensemble, les meilleures. Chlorella vulgaris est celui dont l'utilisation est la moins recommandée.
- . aucun des organismes à l'essai n'a fait preuve de sélectivité (absorption ou rejet) envers l'uranium. À cet égard, les cations et les anions entrent en conflit avec la capacité d'absorption.
- . les solutions de procédés peuvent avoir des caractéristiques, tel un pH faible, qui nécessitent un prétraitement avant la bioabsorption.
- . si on utilise des solutions de procédés, les capacités de bioabsorption sont considérablement plus faibles que celles rapportées pour les solutions de nitrate d'uranyl synthétique ou de chlorure.
- . nous possédons suffisamment de données de base pour concevoir un projet pilote d'installation en usine.

Les recommandations principales de cette étude sont :

- . une évaluation comparative de procédés de récupération par la biomasse par opposition aux technologies de rechange, conventionnelles ou non.
- . des études de base plus approfondies concernant les biopolymères inclus dans le phénomène de bioabsorption et l'identification des structures responsables de la réaction complexe de l'uranium.
- . des études plus approfondies concernant le développement d'un moyen économique de croissance des biomasses en vue de leur production.
- . des recherches plus poussées visant le développement d'une biomasse robuste et inerte qui conserve une capacité d'absorption élevée.
- . de pair avec les essais pilotes, une procédure normalisée pour l'évaluation et le rapport de la capacité de bioabsorption.

EVALUATION OF BIOMASS FOR URANIUM RECOVERY FROM PROCESS STREAMS
STAGE I - EXPERIMENTAL PROGRAM

FINAL REPORT

BY

SENE CONSULTANTS LIMITED

June 1985

Work on this project was conducted under the auspices of the Canada Centre for Mineral and Energy Technology, Energy, Mines and Resources, Canada.

DSS FILE NUMBER : 26SQ.23440-4-9172
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CANMET SCIENTIFIC AUTHORITY: V. Sanmugasunderam

TENTATIVE EXPERIMENTAL WORK SCHEDULE

Evaluation of Biomass for Uranium Recovery From Process Streams

An important criterion in selecting biomass for this study is the ease of cultivation in inexpensive growth medium. The test organism should possess high specific growth rate and high biomass yields in addition to proven uranium adsorption capacity. The study includes one species belonging to the following groups of microorganisms: (Additional species may also be investigated, time and budget permitting)

- a) Actinomycete (e.g. Streptomyces)
- b) Fungal biomass (e.g. Rhizopus)
- c) Yeast biomass (e.g. Saccharomyces)
- d) Algal biomass (e.g. Chlorella)
- e) Mixed culture (e.g. activated sludge)
- f) Microbial cell derivatives (immobilized cell preparations of two of the above cultures based on uranyl ion adsorption capacity and economic considerations).

The specific growth rates and biomass yields of the actinomycete, fungal, and yeast biomass will be evaluated in continuously operating fermentors (chemostat) and the algal biomass in fed-batch growth vessels. Physiologically young and old cells will be derived by variation of the dilution rate (retention time).

Uranium Adsorption Studies - Biological Variables

These studies serve for determining the effect of biomass type, physiological age and viability on the uranyl ion adsorption capacity. The biological variables include:

i) Biomass type

- a) Actinomycete (Streptomyces)
- b) Fungus (Rhizopus)
- c) Yeast (Saccharomyces)
- d) Alga (Chlorella)
- e) Mixed culture (activated sludge)
- f) Immobilized cells (2 types)

ii) Physiological age

- a) young cells
- b) old cells

iii) Viability

- a) viable cells
- b) thermally inactivated cells.

Batch studies will comprise a full factorial design of 28 ($7 \times 2 \times 2$) experiments performed in duplicate.

Column studies involve an actinomycete, fungus, and 2 immobilized cell preparations. There will be a total of 16 column experiments ($4 \times 2 \times 2$).

Both the batch and column studies will be carried out at a constant solid/liquid ratio, at 20°C and a pH of 5.0.

Uranium Adsorption Studies - Process Variables

Process variables to be investigated include temperature, pH and uranyl ion concentration in the process liquid. These process

variables are summarized as follows:

- i) Temperature: 4°C, 20°C, 30°C
- ii) pH : pH = 2, pH = 5, pH = 8
- iii) UO_2^{2+} concentration: - "high" UO_2^{2+} content
- "low" UO_2^{2+} content.

Note: If the pH of the process leach liquor, as received, is sufficiently low, say between pH = 2 and pH = 3, the pH will not be adjusted for determining the pH effect on uranium adsorption at the low pH range.

Batch studies will include a full complement of experiments for each condition as indicated above. Column studies will be performed at with appropriate biomass (fungal biomass and actinomycete) as well as two immobilized cell preparations.

Equilibrium Distribution of Uranium

These experiments are aimed at the determination of the uranium adsorption isotherm. Variables include:

- i) Biomass type (4 biomasses)
- ii) Solid/liquid ratio (4 s/l ratios)
- iii) Low vs high UO_2^{2+} content in the process liquid (2 concentrations).

The experimental conditions (temperature, pH) will depend on the outcome of the previous study. Altogether 32 (4 x 4 x 2) experiments will be performed.

Uranium Desorption Studies

In these studies, the desorption characteristics (desorption rate and equilibria) of the biomass will be investigated. The experimental variables include:

- i) 3 types of biomass (two "best" adsorbing biomass and activated sludge); 2 immobilized cell preparations
- ii) Eluants: sulphuric acid
 ammonium carbonate
 sodium bicarbonate

Each eluant will be examined at two concentrations. Thus, this state of the research program will consist of 30 (5 x 3 x 2) batch studies and 24 (4 x 3 x 2) column studies.

Repeated Adsorption/Desorption Cycles

These experiments will be performed at the best adsorption/desorption conditions as determined in the previous studies. These studies will include the following variables:

- i) Biomass (two suspension cultures and two immobilized cell preparations)
- ii) "high" vs "low" UO_2^{2+} content in process liquid
- iii) Chelating agent (i.e. EDTA).

To study the repeated adsorption/desorption cycling on performance and biomass attrition, each experiment will involve a minimum of 10 cycles. Altogether 8 suspension culture (cyclic batch) and 8 column studies will be performed.

EVALUATION OF BIOMASS FOR URANIUM RECOVERY FROM PROCESS STREAMS
STAGE II - RESULTS - LABORATORY PROGRAM

FINAL REPORT

BY

SENE CONSULTANTS LIMITED

June 1985

Work on this project was conducted under the auspices of the Canada Centre for Mineral and Energy Technology, Energy, Mines and Resources, Canada.

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1.0 INTRODUCTION

1.1 General Background

The ability of microorganisms to extract metal ions from dilute aqueous solutions has been known for over thirty years (Galun et al., 1981). Most studies, however, addressed this phenomenon from a perspective of environmental toxicity. Freshwater and marine algae can accumulate uranium (Sakaguchi et al., 1978; Horikoshi et al., 1981; Sprey and Bochem, 1981), and other heavy metals, to levels that may be toxic to higher aquatic species (Crist et al, 1981). The bioconcentration of toxic heavy metals in sewage sludge imposes restrictions about disposal on agricultural land. Previous research has been summarized recently by the Ontario Research Foundation and Allelix (1984). While concerns about environmental safety were the original impetus for research, several recent studies have dealt with the potential exploitation of microorganisms for bioconcentrating metal values from hydrometallurgical process liquids. This project specifically deals with the application of biomass for uranium recovery from a typical uranium mining process stream.

In Ontario and especially the Elliot Lake area, there are extensive low grade deposits of uranium. Operators such as Denison Mines have found that underground leaching of the ore is becoming increasingly economically attractive, however, the leaching produces vast quantities of low grade process solutions which require concentration underground or pumping to surface. This mine water solution is the type of process stream identified as a potential candidate for the application of biotechnology. Considering that uranium is originally dissolved by enhanced underground leaching with the application of thiobacillus ferrooxidans and related species, it is a logical extension to apply biomass for the uranium recovery.



The enclosed report provides a summary of the current literature, a complete outline of the experimental procedures, results and conclusions and recommendations for future work.

1.2 Terms of Reference

The original statement of work is provided in Appendix A.

1.3 Study Team

The study team included Mr. R. Knapp and Dr. J. Scharer of SENES and Drs. M. Moo-Young, J. Byerley and A. Charles from the Institute of Biotechnology Research, Dept. of Chemical Engineering, University of Waterloo.

SENES Consultants managed the project and co-ordinated the day to day experimental work. The Institute for Biotechnology Research cultured the organisms and undertook all experimental testwork.

1.4 Report Organization

The report structure and content is presented below.

2.0 BACKGROUND INFORMATION - This chapter reviews the literature on mechanism and kinetics of uranium bioadsorption and presents a general discussion on uranium chemistry.

3.0 GROWTH STUDIES AND BIOMASS PRODUCTION - This chapter presents the materials and methods for culturing and harvesting the test organisms.

- 4.0 URANIUM BIOADSORPTION - This chapter presents the experimental procedures for analysis and bioadsorption test work.
- 5.0 RESULTS - GROWTH STUDIES - This chapter presents the basic data on specific growth rates and biomass production and highlights of the problems and successes with fermentation techniques are presented.
- 6.0 RESULTS - BIOADSORPTION TESTWORK - This chapter presents a discussion of the major results of the test work on loading rates, capacities, equilibrium, and desorption and discusses how these are impacted by temperature, pH, physiology and thermal inactivation.
- 7.0 SUMMARY - This chapter presents a summary of the major findings of the report.
- 8.0 CONCLUSIONS AND RECOMMENDATIONS
- 9.0 REFERENCES

APPENDIX A

APPENDIX B

2.0 BACKGROUND INFORMATION

2.1 Mechanisms of Uranium Bioadsorption

Microorganisms are known to exploit various organic macromolecules on their external surfaces (polypeptides, proteins, polysaccharides) to capture essential metal ions from dilute solutions. By electron microscopy, Beveridge and Murray (1976) have shown that wall fragments of Bacillus subtilis accumulate metals to such levels that they can be directly observed by their electron scattering in thin section preparations. Highly adsorbing species particularly (Fe^{3+} and Cu^{2+}) form crystalloid beads along both the inner and the outer surfaces of the cell wall. Partial digestion of the cell wall by lysozyme affects the adsorption of some metal species, while the others remain unaffected. When a series of 12 metal solutions are passed through wall fragments, metal ions such as Fe^{3+} , Ni^{2+} , Ca^{2+} , and Mg^{2+} remain adsorbed, while the others are either displaced or desorbed. On the basis of these results the authors have proposed the existence of select sites on the cell wall surface for the various metal ions.

The metal ions scavenged from the environment are eventually transported across the cytoplasmic membrane by specialized carrier molecules. These carrier molecules possess a cage-like interior to accommodate the metal ion; while the hydrophobic exterior imparts solubility in the lipid-rich cytoplasmic membrane (Lehninger, 1982). This process, known as facilitated diffusion, is to satisfy the requirements for synthesizing various metalloproteins essential for metabolic functions. Nakajima et al. (1981) have shown, for example, that 95.8% of the Cu^{2+} adsorbed from dilute (0.7 mg/L) copper solutions occurs in the membrane or intracellularly in Chlorella regularis cells after 1 hr. incubation. Some bacterial species, notably the

genera Sphaerotilus and Leptothrix (Mulder, 1974), accumulate copious amounts of iron and manganese hydroxides extracellularly. These hydroxides are deposited in sheaths that serve primarily as a protective structure for the microorganisms.

Unlike the essential metal ions discussed above, the uranyl ion and its derivatives are not known to function in any metal-enzyme complex. Yet, the bioadsorption of uranium has been demonstrated in a variety of microorganisms including bacteria, fungi, yeasts, and algae. This phenomenon has been observed in both natural systems and under artificial conditions in the laboratory. The data base on uranium bioaccumulation in freshwater biota has been reviewed by Blaylock (1982). The most complete set of data available is for Lake Michigan. In this lake, the ^{238}U bioaccumulation factor (BF) ranges from 88-145 mL/g (fresh weight) in phytoplankton to 0.3 - 0.7 mL/g (fresh weight) in piscivorous and omnivorous fish. Differences in bioaccumulation exist among the members of the phytoplankton community. Sakaguchi et al. (1978) have observed that the uptake of uranium by freshwater microalgae from 1 mg/L uranium solution (pH=8.0) is the highest in Chlorella regularis cultures (3.93 mg/g dry weight) followed by various Chlamydomonas (2.33-3.40 mg/g dry weight) and Scenedesmus (0.80-1.92 mg/g) species.

A definitive comparison of other protists with algae is difficult and tentative at best, since conditions for bioadsorption varied amongst the investigators. In general, actinomyces (Streptomyces levoris, Streptomyces viridochromogenes) appear to have the highest uranium bioadsorption capacities (Horikoshi et al., 1981), followed by bacteria such as Pseudomonas aeruginosa (Horikoshi et al., 1981, Strandberg et al., 1981), Zooglea ramigera (Norberg and Persson, 1984), municipal sludge (Tsezos and Volesky, 1981), Thiobacillus ferrooxidans (DiSpirito et al., 1983), the yeast Saccharomyces cerevisiae (Horikoshi et al., 1981)

and the fungi including Aspergillus niger and Aspergillus terreus (Tsezos and Voleski, 1981) in decreasing order.

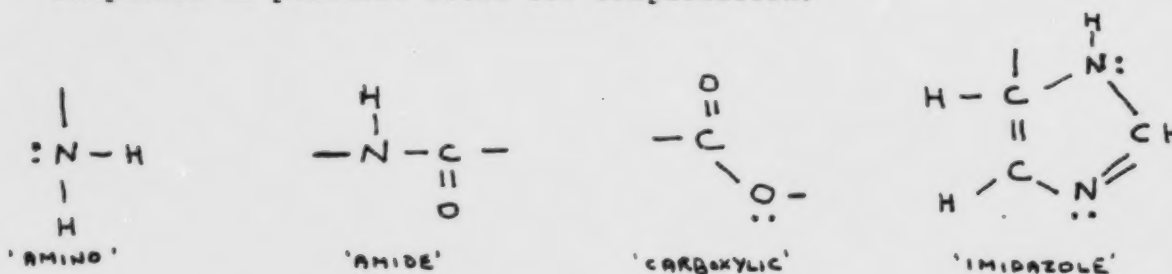
Fungi belonging to the genera Rhizopus and Penicillium have been found to be the most notable exceptions of the above order (Tsezos and Voleski, 1981) in as much as the maximum uranium adsorption capacities of P. Chrysogenum (165 mg U/g biomass) and R. arrhizus (>180 mg U/g biomass) exceeded all other species.

As in cases of essential metal ions, the components of the cell wall act as collectors of the uranyl ion (UO_2^{2+}). Electron micrographs of Pseudomonas aeruginosa, Saccharomyces cerevisiae (Strandberg et al., 1981) and Rhizopus arrhizus (Tsezos and Voleski, 1982) show electron dense, crystalloid uranium deposits (presumably uranium oxides or hydroxides) in the cell wall after exposure to uranium solution. The formation of these uranium granules is reversible as reflected by the disappearance of the granules in electron micrographs taken after uranium desorption by suitable reagent viz. NaHCO_3 (Tsezos, 1984). There is some evidence that the transport of UO_2^{2+} into the cell interior can also take place. Over 90% of UO_2^{2+} adsorbed from weak solutions (1 mg/L U) reside in the cytoplasm of Chlorella regularis (Nakajima et al, 1980) and Synechococcus elongatus (Horikoshi et al, 1979) after 1 hour of incubation. Berthelin and Munier-Lamy (1983) have interpreted the dependence of uranium uptake on growth conditions of viable Aspergillus ochraceus and Penicillium funiculosum cells that retention by wall components is by no means the only significant process. Rather, the entire energetics processes of cells, in particular, the dissipation of the proton motive force could be also involved. DiSpirito et al. (1983) have found that, of the 734 mg U/g cell protein adsorbed from 10 mM UO_2^{2+} solutions, 28.5% is associated with the cell wall, 17.5% with the cytoplasmic membrane, while only 4% of the uranium is located in the cytoplasm. It is interesting to note



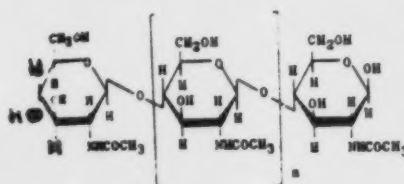
that, in cells killed with 10 mM KCN the adsorption capacity increases to 110.8 mg U/g cell protein, the increase in cytoplasmic uranium retention (21.6%) being the most significant factor.

The nature of the interaction between the uranyl ion and the constituents of the cell wall on the molecular level is not well understood. Crist et al. (1981) have suggested the following functional groups found in proteins and protein-polysaccharide complexes as possible sites for complexation:



Of the above functional groups, the carboxyl carries a net negative charge, amino and imidazole groups are positively charged, while the amide group is uncharged at physiological pH conditions (pH of 4.5 to 9.0). In some cell wall polysaccharides, phosphate, and sulphate residues can provide additional binding sites.

Tsezos (1983) has suggested that the uranyl ion uptake by the cell wall is due to complexation with chitin nitrogen. Chitin is a polysaccharide consisting mainly of unbranched chains of N-acetyl-glucosamine



The deacetylated form of chitin (poly-N-glucosamine) is known as

chitosan. Fungal chitin tends to be a mixture of N-acetyl-glucosamine (chitin) and N-glucosamine (chitosan) residues. In bacterial cell walls, a polysaccharide is formed by units of N-acetyl-glucosamine and muramic acid. Muramic acid is also an acetylamino sugar with a lactate residue on the C-3 position.

The mechanism of uranium bioadsorption, as proposed by Tsezos and Volesky (1982a) is as follows. First, the uranium species, presumably the uranyl ion, coordinates with the amino nitrogen residue (glucosamine) or the amide nitrogen (acetyl-glucosamine) in chitinous crystallites. Then, additional uranium uptake occurs at the proximity of the initial adsorption sites. Finally, the uranyl ion hydrolyzes to uranyl hydroxide ($\text{UO}_2(\text{OH})_2$) and forms the characteristic uranium precipitates seen in the electron micrographs. While the initial adsorption process is fast, (60 seconds or less), crystal growth may continue for 30 minutes or more. In Rhizopus arrhizus cells, approximately 60% of the uranium is adsorbed initially.

Although Tsezos and Volesky (1982a, 1982b) have provided plausible evidence by x-ray energy dispersion analysis and IRT spectroscopy, research by other investigators casts some doubt on a chitin-based uranium bioadsorption model. Pure chitin preparations are not particularly good adsorbers of uranium. In fact, the maximum adsorption capacity of chitin has been 8.8 mg U/g dry weight as compared with 180 mg U/g dry weight with Rhizopus arrhizus cells (Tsezos, 1983). From the data of Galun et al. (1982) it appears that uranium retention by chitin is not substantially better than by carboxymethyl cellulose. The latter has similar structure, but contains no nitrogen residues. It has been shown (Strandberg et al., 1981) that the uranyl ion does not complex with either the amino groups of monocarboxylic acids (serine, glycine) or the amide group of N-substituted dicarboxylic acids (glutamine, for instance). However, the

distant carboxylic group of dicarboxylic acids (glutamic acid) forms complexes freely with the uranyl ion. Complexation and precipitation with inorganic ammonium ion (ammonium diuranate) occur at alkaline pH conditions only.

Sakaguchi et al. (1981) has shown that uranium adsorption improves if chitin or chitosan is phosphorylated. Chitin and chitosan phosphates adsorbed 105-110 mg U/g dry weight at pH=5 (optimum pH) in comparison with less than 10 mg U/g dry weight in non-phosphorylated samples. Were the amino groups involved in complexation with the uranyl ion, metal ions that are known to form strong complexes with organic amines (Cu^{2+} for example, see Crist et al. 1981) would compete successfully with the UO_2^{2+} ion for adsorption sites. However, Cu^{2+} has had no significant affect on UO_2^{2+} uptake at the highest concentration of copper tested (100 ppm Cu^{2+}), while in the presence of Fe^{3+} , UO_2^{2+} adsorption is reduced ten fold in comparative uranium uptake studies by Penicillium mycelial biomass (Galun et al., 1984). Similarly, ammonium carbonate is only marginally better desorbing agent for adsorbed uranium than sodium bicarbonate (Galun et al, 1983).

These observations would preclude the significant, direct participation of "amino" and "amide" groups of chitin or cell mass proteins in UO_2^{2+} complexation reactions. Indeed, the observations point toward negatively charged residues such as carboxylic (Strandberg et al., 1981) and phosphate groups (Tobin et al., 1984; Sakaguchi et al, 1981) as complexing agents. It is evident that substantially more basic research is needed. Such research would provide a more systematic basis to test various biomasses than the trial and error screening approach presently practiced.

2.2 Uranium Adsorption Equilibrium and Kinetics

Many separation processes, both natural and anthropogenic, are based upon the partitioning of solutes between two or more phases. In a closed system and in numerous open systems, this partitioning will approach the state of thermodynamic equilibrium. The equilibrium distribution is, of course, dependent on the phase-rule variables such as temperature, pressure, pH, ionic strength, etc. In 1907, Freundlich was the first to describe the distribution of a solute between a solid and a liquid phase by a mathematical expression:

$$X_{eq} = A C_{eq}^n \quad (2.2.1)$$

where:

X_{eq} = equilibrium concentration of the solute on the solid phase

C_{eq} = equilibrium concentration of the solute in the liquid phase

A, n = characteristic constants for the equilibrium isotherm.

Equation 3.2.1, known as the Freundlich isotherm, is essentially an empirical expression. A mechanistic adsorption model was published in 1917 by Langmuir. The Langmuir isotherm takes into account a finite number of adsorption sites on the solid surface, each site being capable of sequestering a solute from the solution. This approach leads to the following equilibrium expression:

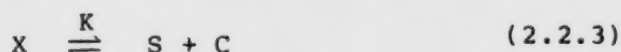
$$X_{eq} = X_{max} \frac{C_{eq}}{K + C_{eq}} \quad (2.2.2)$$

where

X_{eq} = equilibrium concentration of the solute on the solid surface

C_{eq} = equilibrium concentration of the solute in the liquid phase
 X_{max} = maximum attainable concentration of the solute on the solid surface
 K = half saturate constant

In the Langmuir isotherm (Equation 2.2.2) the parameters X_{max} and K have been assigned physical significance. The maximum adsorption capacity, X_{max} , signifies a theoretical adsorption resulting from all adsorbing sites capturing a solute molecule. The mechanistic interpretation of the half saturation constant, K , is that it represents the dissociative equilibrium between any adsorbing site(s) and a solute molecule C;



$$K = \frac{[S][C]}{X} \Big|_{eq}$$

The units of K are identical with the units of solute in the liquid phase. The Freundlich and the Langmuir isotherms were the basis of developing numerous other equilibrium expressions. A generalized isotherm for multicomponent systems, which is perhaps, most often quoted in the recent literature, has been proposed by Fritz and Schluender (1974). Although, this isotherm was originally developed for adsorbing organic solutes, its use for other systems is non-restrictive.

The algebraic expression for the Fritz-Schluender isotherm is as follows:

$$X_i = \frac{a_{i0} C_i^{b_{i0}}}{c_i + \sum_{j=1}^n a_{ij} C_j^{b_{ij}}} \quad (2.2.4)$$

where

X_i = the amount of solute "i" adsorbed per unit weight of solid

c_i = equilibrium concentration of the "i"th component in the liquid

n = total number of components

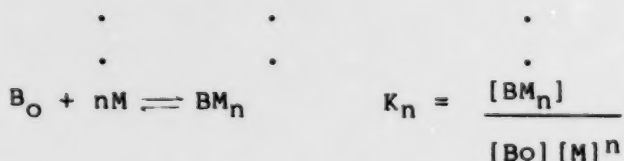
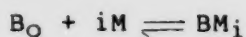
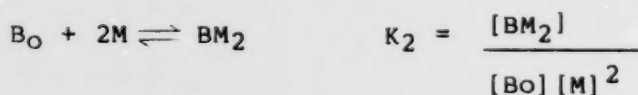
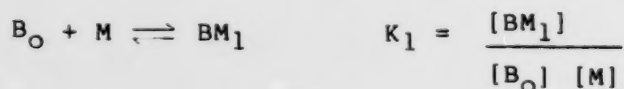
$a_{io}, a_{ij}, b_{io}, b_{ij}, c_i$ = characteristic constants. The above equation includes some of the well known isotherms as special cases. The number of non-linear parameters to be estimated, however, would limit its general use in many, often incompletely defined system.

Benjamin and Leckie (1981) have derived a conceptual adsorption model for metal-ligand-surface interactions. In particular, they have considered the effect of ligand formation in the liquid phase, both organic ligands and inorganic ligands, on the adsorption capacity for a metal species. Their approach was based on the development of a series of equilibrium expressions, similar to the Langmuirian approach (see Equation 2.3.2), involving proton (hydrogen ion) exchange, free metal adsorption, and metal ligand adsorption on the solid surface. The authors have shown that the complexation of the metal ion with either inorganic or organic ligands dramatically alters the adsorption capacity. The adsorption of "metallike" species tends to decrease, while the adsorption of "ligandlike" molecules tends to increase with increasing pH. A rather interesting feature of the adsorption profiles is that the fraction of "ligandlike" species adsorbed is relatively independent of the adsorbate concentration. On the other hand, the fractional adsorption of "metallike" molecules is strongly influenced by the adsorbate level.

The adsorption of small molecules by biological matter is a common phenomenon. In fact, the major function of many

biopolymers has been considered to be the binding of ions or ligands. It is not surprising, therefore, that the mechanisms of binding reactions have been under intensive study by numerous investigators. In most cases, the binding involves a non-covalent bond formation between the ion (or ligand) and a specific region of the macromolecule. This region of the macromolecule is often called the binding site. Since complexation may involve conformational changes of the macromolecule and interaction between two or more sites, the sequestering process may become complex. The binding may be so strong, that the process appears to be essentially irreversible. If no covalent bond formation takes place, however, the complexation can be best described as an equilibrium process. All equilibrium processes are inherently reversible.

In the ensuing analysis, the interaction between a bio-polymer (B) having "n" binding sites for a metal species (M) is considered. This process can be formulated as a series of equilibrium expressions involving the biopolymer and "i" number of metal ions as follows (van Holde, 1985):



where

$$[] = \text{concentration (moles/L)} \quad (2.2.5)$$

Applying the equilibrium expressions above, the moles of metal ions captured per mole of biopolymer becomes the following:

$$= \frac{\sum_{i=1}^n i [BM_i]}{\sum_{i=0}^n [BM_i]} = \frac{\sum_{i=1}^n i K_i [M]^i}{1 + \sum_{i=1}^n K_i [M]^i} \quad (2.2.6)$$

It can be easily shown, that for $n=1$, equation (2.2.6) reduces to the Langmuir isotherm. For biopolymers having multiple adsorption sites, the number of possible isomers having a particular complex state $[BM_i]$ is given by the permutation statistic:

$$n_{i,n} = \frac{n!}{(n-i)! i!} \quad (2.2.7)$$

If the sites are non-interacting (non-cooperative) and the equilibrium constant, K_i , is identical for each isomer having the configuration BM_i , equation (2.2.6) can be simplified to yield:

$$= \frac{nK [M]}{1 + K [M]} = n \frac{[M]}{1/K + [M]} \quad (2.2.8)$$

$$K = \prod_{i=1}^n K_i^{1/n}$$

If the number of moles of adsorbing biopolymer per g of biomass is constant, say " γ ", multiplying Equ. (2.2.8) by " γ " yields an

expression analogous to the Langmuir isotherm:

$$X_{eq} = X_{max} \frac{[M]}{K + [M]} \quad (2.2.9)$$

where

$$X_{eq} = \gamma \nu$$

$$X_{max} = n$$

$$K = 1/K$$

In equations 2.2.8 and 2.2.9 the binding is assumed to be fully random and the equilibrium constants (K_i 's) are to follow a regular pattern:

$$K_i = K_1^i \quad (2.2.10)$$

Interaction, i.e. cooperation, between sites gives rise to a more complex isotherm known as the Aldair equation:

$$= \frac{\sum_{i=1}^n i \frac{n! K_i}{(n-i)! i!} [M]^i}{1 + \sum_{i=1}^n \frac{n! K_i}{(n-i)! i!} [M]^i} \quad (2.2.11)$$

$$K_i \neq K_1^i$$

The quantitative aspects of the uranium bioadsorption equilibrium has been seldom addressed in previous studies. Crist et al. (1981) have noted that the Langmuir isotherm (Equation 2.2.2) is applicable to describe the equilibrium uptake of uranium by fungi, but no details were provided. Tzesos and Volesky (1981) have compared the use of the Freundlich isotherm (Equation 2.2.1) with the Langmuir isotherm for uranium bioadsorption at pH values of 2.0 and 4.5 by various microorganisms including bacteria,

fungi and sewage sludges. Calculating the residual sum of squares, the authors have concluded that the Freundlich isotherm is marginally better than the Langmuir isotherm. However, the differences between the goodness of fit of the two models do not appear to be statistically significant, the average ratio of the residual sum of squares (Langmuir isotherm/Freundlich isotherm) is 1.14 and 1.15 at pH = 4.5 and pH = 2.0, respectively (7 biomass types). From the pH effects on bioadsorption, the uranyl ion (UO_2^{2+}) has been identified as the principal species being adsorbed. It appears that the phenomenon of bioadsorption is not dependent on the solubility of U(VI) compounds.

The kinetics of the uranium bioadsorption has not been studied quantitatively in the past. In view of the lack of previous model development, a plausible kinetic model for metal ion bioadsorption at non-cooperative adsorption sites is offered herein. It can be shown, that the rate of metal ion adsorption expressed as moles/L ion adsorbed per unit time, is given by the following differential equation:

$$\frac{d \sum_{i=0}^n i[\text{BM}_i]}{dt} = k_{(+)} \left[\sum_{i=0}^n [\text{BM}_i] - \sum_{i=0}^n i[\text{BM}_i] \right] [\text{M}] - k_{(-)} \sum_{i=0}^n i[\text{BM}_i] \quad (2.2.12)$$

where:

$[\text{BM}_i]$ = molar concentration of biopolymer with "i" adsorbed metal ion

$\sum_{i=0}^n i[\text{BM}_i]$ = molar concentration of adsorbed metal ion.

$n \sum_{i=0}^n [BM_i]$ = molar concentration of total (potentially available) adsorption sites.

n = number of adsorption sites per biopolymer

$k_{(+)}$ = reaction rate constant in the forward (adsorption) direction

$k_{(-)}$ = reaction rate constant in the reverse (desorption) direction

If i is the concentration of biomass (g/L dry weight) and K the dissociative equilibrium constant, the adsorption capacity (mg metal adsorbed/g biomass), the maximum adsorption capacity, and the reaction rate constant in the reverse direction are defined as follows:

$$x = \frac{\sum_{i=0}^n i [BM_i]}{\sigma}; X_{\max} = \frac{\sum_{i=0}^n [BM_i]}{\sigma}; k_{(-)} = K k_{(+)} \quad (2.2.13)$$

where:

x = mg of U adsorbed/g biomass

$X_{\max}$ = maximum uranium uptake capacity (mg U/g biomass)

Substituting the terms defined in Equation 2.2.13 into Equation 2.2.12 yields the following differential equation:

$$\frac{dx}{dt} = k_{(+)} \{ (X_{\max} - x) C - Kx \} \quad (2.2.14)$$

where:

c = concentration of uranium in liquid (mg/L)



If the volume occupied by the dry biomass is negligible, as is the case in most studies, the mass balance for uranium on a unit volume basis is as follows:

$$C_0 = \sigma x + C \quad (2.2.15)$$

where:

C_0 = initial (total) concentration of uranium (mg/L)

X = uranium bioadsorption capacity (instantaneous) (mg U/g biomass)

C = concentration of uranium in the liquid phase (mg/L)

Recognizing that, from Equation 2.2.15, $\frac{\sigma dx}{dt} = - \frac{dc}{dt}$, after proper substitutions, Equations (2.2.14) may be integrated analytically to give the following expression for the time dependent uranium concentration in the liquid phase:

$$C = \frac{1}{2} \frac{(\alpha - \beta) + (\alpha + \beta) \frac{2C_0 + \beta - \alpha}{2C_0 + \beta + \alpha} e^{-\alpha k_{(+)t}}}{1 - \frac{2C_0 + \beta - \alpha}{2C_0 + \beta + \alpha} e^{-\alpha k_{(+)t}}} \quad (2.2.16)$$

where:

$$= [4KC_0 + (\sigma X_{\max} - C_0 + K)^2]^{1/2}$$

$$= \sigma X_{\max} - C_0 + K$$

C = concentration of uranium in the liquid phase at any time, t (mg U/L)

C_0 = initial concentration of uranium (mg U/L)

Equation (2.2.16) is the exact solution to the differential Equation (2.2.14). Due to the highly non-linear nature of the parameters and the limited kinetic data available, its use is restricted to some special cases in the present study. If the



adsorption is rapid in comparison to the experimental time frame, the following empirical equation, based on analogy to interphase mass transfer, may be used as an approximation of the bioadsorption kinetics:

$$\frac{dx}{dt} = k (X_{eq} - X) \quad (2.2.17)$$

where

k = empirical reaction rate constant (hr^{-1})

X_{eq} = equilibrium bioadsorption capacity (mg U/g biomass)

X = instantaneous bioadsorption capacity (mg U/g biomass)

Equation 2.2.17 can be readily integrated to yield:

$$X = X_{eq} (1 - e^{-kt}) \quad (2.2.18)$$

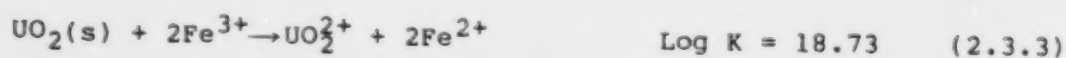
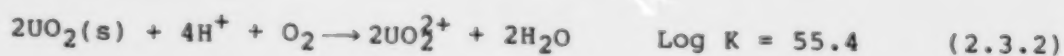
Strictly speaking, Equation (2.2.18) is an asymptotic solution of the original differential equation (Equation 2.2.14) when the bioadsorption capacity approaches its equilibrium value. Thus, if the bioadsorption is rapid, Equation (2.2.18) is expected to give a good estimate of the equilibrium position for a given set of initial conditions. The empirical rate constant, k , is a relative measure of the rate of approach to equilibrium.

2.3 Chemistry of Uranium Process Solutions

Although several oxidation states of uranium are known, only U(IV) and U(VI) are usually found under natural conditions as might be expected in a uranium mine environment. Uranium in other oxidation states, for example aqueous solutions of U(V), disproportionate to U(IV) and U(VI) species (Ames and Rai 1978):



The solution chemistry of uranium minerals and the formation of aqueous complexes have been reviewed by Langmuir (1978), Ames and Rai (1978) and Lemire and Tremaine (1980). In the presence of oxygen (for example, in an underground biological leaching stope) U(IV) minerals are unstable and are oxidized to uranyl ion. In an acidic environment, either oxygen or ferric iron serve as oxidants according to the following reactions:



At the typical contaminant concentrations measured in the acidic mine water from the Elliot Lake tailings area (Table 6.1), oxidation by reaction mechanism (2.3.2) is thermodynamically favoured. The rate of uranyl (UO_2^{2+}) ion formation however, may be higher by reaction (2.3.3).

The solubility products of the common U(VI) minerals are summarized in Table 2.3.1. The solubility products (K_{sp}) for the oxides have been obtained from data provided by Lemire and Tremaine (1980). Solubility products for autinites (uranyl phosphates) were calculated from Gibbs free energy data compiled by Langmuir (1978) and Stumm and Morgan (1981). The data for the vanadates have been obtained from Langmuir (1978). Uranyl vanadates, particularly carnotite ($\text{K}_2(\text{UO}_2)_2(\text{VO}_4)_2$), are the least soluble U(VI) minerals. In general, autinites appear to follow in solubility, while uranyl oxides and hydroxides are the most soluble minerals.

At pH values of 3.0 and below, which are common to acid mine waters, none of the U(VI) minerals are stable and equilibrium UO_2^{2+} concentrations exceed 500 mg.L^{-1} . In addition, UO_2^{2+} readily complexes to form soluble complexes with hydroxide (OH^-),

carbonate (CO_3^{2-}) and sulphate (SO_4^{2-}). The most important complexation reactions are summarized in Table 2.3.2. The formation of these inorganic complexes greatly enhances the solubility of U(VI) minerals. In an acidic environment at pH values of 3.0 and below, UO_2^{2+} , UO_2SO_4 (aq) and $\text{UO}_2(\text{SO}_4)_2^{2-}$ are the predominant species. At pH values near neutrality, $\text{UO}_2(\text{OH})^+$, $\text{UO}_2(\text{OH})_2$ (aq) and the sulphate complexes (UO_2SO_4 , $\text{UO}_2(\text{SO}_4)_2^{2-}$) become the most important ligands, while in the alkaline pH range, the anionic carbonate complexes ($\text{UO}_2(\text{CO}_3)_2^{2-}$, $\text{UO}_2(\text{CO}_3)_3^{4-}$) are present in significant concentrations. The latter complexes explain the use of bicarbonate solutions for uranium desorption at alkaline pH.

TABLE 2.3.1

Dissolution Equilibria of U(VI) Minerals

<u>Dissolution Reaction</u>	<u>Equilibrium constant at 25°C (log K_{sp})</u>
$-\text{UO}_3 + 2\text{H}^+ = \text{UO}_2^{2+} + \text{H}_2\text{O}$	7.7
$-\text{UO}_2(\text{OH})_2 + 2\text{H}^+ = \text{UO}_2^{2+} + 2\text{H}_2\text{O}$	5.7
$\text{UO}_3 - 2\text{H}_2\text{O} + 2\text{H}^+ = \text{UO}_2^{2+} + 3\text{H}_2\text{O}$	5.6
$2\text{U}_3\text{O}_8 + 12\text{H}^+ + \text{O}_2 = 6\text{UO}_2^{2+} + 6\text{H}_2\text{O}$	70.0
$\text{H}_2(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + 2\text{H}^+ + 2\text{PO}_4^{3-}$	-48.2
$\text{Na}_2(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + 2\text{Na}^+ + 2\text{PO}_4^{3-}$	-34.5
$\text{K}_2(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + 2\text{K}^+ + 2\text{PO}_4^{3-}$	-35.4
$(\text{NH}_4)_2(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + 2\text{NH}_4^+ + 2\text{PO}_4^{3-}$	-39.1
$\text{Mg}(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + \text{Mg}^{2+} + 2\text{PO}_4^{3-}$	-31.2
$\text{Ca}(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + \text{Ca}^{2+} + 2\text{PO}_4^{3-}$	-31.5
$\text{Ba}(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + \text{Ba}^{2+} + 2\text{PO}_4^{3-}$	-31.7
$\text{Fe}(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + \text{Fe}^{2+} + 2\text{PO}_4^{3-}$	-31.8
$\text{Cu}(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + \text{Cu}^{2+} + 2\text{PO}_4^{3-}$	-32.2
$\text{Pb}(\text{UO}_2)_2(\text{PO}_4)_2 = 2\text{UO}_2^{2+} + \text{Pb}^{2+} + 2\text{PO}_4^{3-}$	-31.8
$\text{K}_2(\text{UO}_2)_2(\text{VO}_4)_2 = 2\text{UO}_2^{2+} + 2\text{K}^+ + 2\text{VO}_4^{3-}$	-56.9
$\text{Ca}(\text{UO}_2)_2(\text{VO}_4)_2 = 2\text{UO}_2^{2+} + \text{Ca}^{2+} + 2\text{VO}_4^{3-}$	-53.4

TABLE 2.3.2

Uranium (VI) Complexation ReactionsSignificant reactions at pH $\leq$ 6.0

<u>Reaction</u>	<u>Equilibrium Constant (Log K)</u>
$\text{UO}_2^{2+} + \text{H}_2\text{O} = \text{UO}_2(\text{OH})^+ + \text{H}^+$	-5.8
$\text{UO}_2^{2+} + 2\text{H}_2\text{O} = \text{UO}_2(\text{OH})_2(\text{aq}) + 2\text{H}^+$	-12.1
$2\text{UO}_2^{2+} + 2\text{H}_2\text{O} = (\text{UO}_2)_2(\text{OH})_2^{2+} + 2\text{H}^+$	-5.6
$\text{UO}_2^{2+} + \text{CO}_3^{2-} = \text{UO}_2\text{CO}_3(\text{aq})$	10.1
$\text{UO}_2^{2+} + 2\text{CO}_3^{2-} = \text{UO}_2(\text{CO}_3)_2^{2-}$	17.1
$\text{UO}_2^{2+} + 3\text{CO}_3^{2-} = \text{UO}_2(\text{CO}_3)_3^{4-}$	21.4
$\text{UO}_2^{2+} + \text{SO}_4^{2-} = \text{UO}_2\text{SO}_4(\text{aq})$	2.72
$\text{UO}_2^{2+} + 2\text{SO}_4^{2-} = \text{UO}_2(\text{SO}_4)_2^{2-}$	4.17
$\text{UO}_2^{2+} + \text{HPO}_4^{2-} + \text{H}^+ = \text{UO}_2\text{H}_2\text{PO}_4^+$	10.2
$\text{UO}_2^{2+} + 2\text{HPO}_4^{2-} + 2\text{H}^+ = \text{UO}_2(\text{H}_2\text{PO}_4)_2(\text{aq})$	19.9

3.0 GROWTH STUDIES AND BIOMASS PRODUCTION

3.1 Organisms and Growth Media

Altogether 7 microbial biomasses were used in the uranium bioadsorption studies. These included the following:

Bacteria

Streptomyces levoris ATCC #15876

Mixed culture (activated sludge)

Yeast

Saccharomyces cerevisiae

Fungi

Chaetomium cellulolyticum

Rhizopus arrhizus ATCC #6204

Rhizopus oligosporus ATCC #22959

Alga

Chlorella vulgaris ATCC #30581

Stock cultures listed with ATCC deposition numbers were acquired from the American Type Culture Collection, Bethesda, Md., USA; Saccharomyces cerevisiae and Chaetomium cellulolyticum were obtained from the University of Waterloo, Institute for Biotechnology Research, Process Development Center, while activated sludge was obtained from the Waterloo Pollution Control Plant. Stock cultures were maintained and inocula were prepared under the direction of Prof. A. M. Charles, Dept. of Biology, University of Waterloo.

Four of the above cultures were grown routinely during the course of this study. These were the fungus, Rhizopus arrhizus, the yeast Saccaromyces cerevisiae, the actinomyces Streptomyces levoris and the alga Chlorella vulgaris. For purposes of reproducibility, activated sludge was incubated for 24 hours

after receiving from the sewage treatment plant. A brief description of each growth medium is provided.

Rhizopus was grown in the medium suggested by Tobin et al, 1984, consisting of sucrose, peptone, tryptone, KH_2PO_4 , NaNO_3 and MgSO_4 at a pH of 6. The culture was also successfully grown in a medium containing potato peels, glucose and KH_2PO_4 . Incubation was carried out at 30°C for a period of 72 hours.

Streptomyces was cultured in medium containing glucose, tryptose, phosphate and ferric chloride. Growth studies were conducted on a less expensive medium containing sucrose, peptone, KNO_3 , K_2HPO_4 , NaCl and MgSO_4 . The pH was adjusted to 7 and incubated at 30°C for 96 hr.

Saccharomyces was grown in a medium containing glucose, yeast extract, urea, $(\text{NH}_4)_2\text{SO}_4$, K_2HPO_4 , KH_2PO_4 , MgSO_4 and CaCl_2 . The medium pH was adjusted to 5.0 and incubated at 30°C for 30 hr.

Chlorella was grown heterotrophically in the dark on a modified Bristol solution containing glucose, yeast extract, NaNO_3 , KH_2PO_4 , K_2HPO_4 , CaCl_2 , NaCl , MgSO_4 EDTA and several trace metals. the pH was adjusted to 7.0 and incubated at 30°C for 6 days.

A summary of the growth medium composition for each biomass is provided in Table 3.1.1.

The two additional fungi, Chaetomium cellulolyticum and Rhizopus oligosporus were used in some experiments only for comparative purposes. It should be noted, that these organisms were not described in the contract for this work.

TABLE 3.1.1

GROWTH MEDIA

i) Rhizopus arrhizus ATCC #6204

Rhizopus oligosporus ATCC #22959

Medium #1

<u>Constituent</u>	<u>Concentration (g/L)</u>
Sucrose	20
Peptone	5
Tryptone	5
KH ₂ PO ₄	1
NaNO ₃	1
MgSO ₄ -7H ₂ O	0.5

pH = 6.0

Medium #2

Potatoes	300
Glucose	20
KH ₂ PO ₄	1

ii) Saccharomyces cerevisiae

<u>Constituent</u>	<u>Concentration (g/L)</u>
Glucose	10
Yeast extract	1
Urea	0.4
(NH ₄) ₂ SO ₄	1.4
K ₂ HPO ₄	1
KH ₂ PO ₄	1
MgSO ₄ · 7H ₂ O	0.2
CaCl ₂	0.1

pH = 5.0 Adjusted with H₂SO₄

iii) Streptomyces levoris ATCC #15876

Medium #1

<u>Constituent</u>	<u>Concentration (g/L)</u>
Sucrose	10-20
Peptone	20
KNO ₃	3
K ₂ HPO ₄	1
NaCl	0.5
MgSO ₄ · 7H ₂ O	0.5
pH = 7.0	

Medium #2

Glucose	10
Tryptose phosphate	15
FeCl ₃ · 6H ₂ O	0.001

iv) Chlorella vulgaris ATCC #30581

<u>Major</u>		<u>Trace</u>	
<u>Constituents</u>	<u>Conc. (g/L)</u>	<u>Constituents</u>	<u>Conc. (mg/)</u>
Glucose	5.00	FeCl ₃ · 6H ₂ O	5
Yeast Extract	1.00	H ₃ BO ₃	11
NaNO ₃	0.25	ZnSO ₄ · 7H ₂ O	9
K ₂ HPO ₄	0.075	MnCl ₂ · 4H ₂ O	1.4
KH ₂ PO ₄	0.175	Na ₂ MoO ₄ · 4H ₂ O	0.7
CaCl ₂ · 2H ₂ O	0.025	CuSO ₄ · 5H ₂ O	1.6
NaCl	0.025	CoCl ₂ · 6H ₂ O	0.5
MgSO ₄ · 7H ₂ O	0.075		
EDTA	0.0500		

pH = 7.0 Adjusted with H₂SO₄ or KOH

3.2 Fermentation Equipment and Harvesting Procedures

The fermentation equipment included a 20L total volume (15L working volume) Marubishi fermentor for batch growth experiments, a 40L (30L working volume) air lift fermentor for some batch and all continuous growth studies and a 4L algal growth vessel for Chlorella cells only.

A photograph of the Marubishi fermentation system is shown on Figure 3.2.1. The fermentation vessel is fully baffled, mechanically agitated, and jacketed for temperature control. The medium was sterilized in situ by heating with steam through the jacket. The vessels were inoculated with 10% (by volume) cultures of the test organism. During the course of the fermentations, the agitation was maintained at 500 RPM and aerated at a rate of 10L air (STP) per minute. Samples were routinely examined under light microscope for purity.

The air lift fermentor is shown on Figure 3.2.2. The vessel consisted of a Pyrex glass column equipped with a slanted conical bottom. The slanted bottom design was to induce good circulation at relatively low air flow rates. Air was sparged at the bottom of the conical section. The medium was sterilized by direct steam injection. Temperature control was accomplished by a heating tape surrounding the vessel. Ports for inoculum and sampling were located on the top stainless steel plate and sterilized by open flame. Aeration during fermentations was maintained at 10-50L/min (STP), depending on the volume of the fermentation broth (continuously fed-batch studies) and the rheological properties of the biomass. Sterile air was supplied through a disposable filter module. In continuously fed experiments, sterile medium was maintained under pressure in the stainless steel tanks shown on Figure 3.2.2 and metered to the air-lift fermentor as desired. Figure 3.2.2 shows the typical

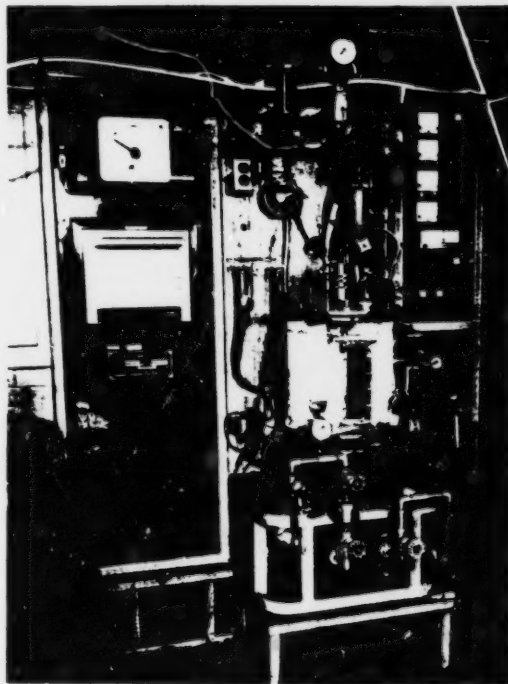


Figure 3.2.1 - Marubishi Fermentor

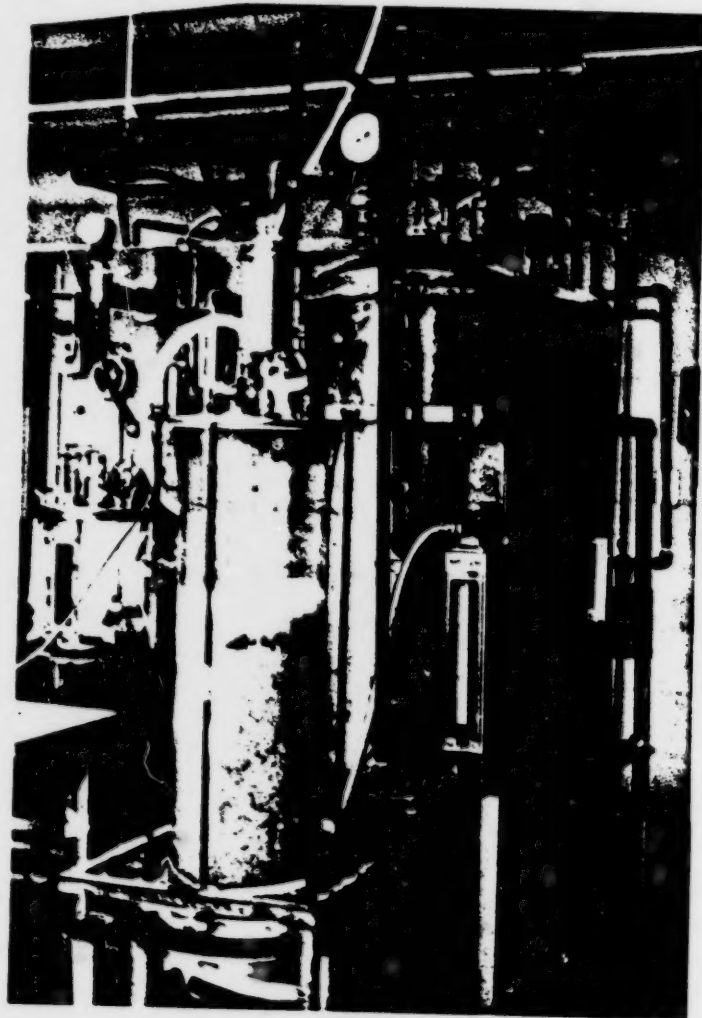


Figure 3.2.2 Air-Lift Fermentor



Figure 3.2.3 Rhizopus Growth in Air-Lift Fermentor

consistency of Rhizopus arrhizus grown in the unit.

Fresh biomass was produced at approximately 2-week intervals during the entire course of this study. The biomass was harvested by filtration. Cultures of Rhizopus were the easiest to harvest by rapid filtration. The harvesting of Streptomyces and Saccharomyces were often time consuming due to rapid clogging of the filter surface. Activated sludge and Chlorella filtered more easily. The biomasses were stored as pastes at 4°C. The storage life was approximately 2 weeks.

3.3 Batch Growth Experiments

As an integral part of this report, batch growth experiments were performed with the following microorganisms.

Rhizopus arrhizus

Streptomyces levoris

Saccharomyces cerevisiae

Chlorella vulgaris

It should be noted, that Chlorella vulgaris has been substituted for the originally proposed species, Chlorella regularis. Chlorella regularis has been reported to be a good metal adsorbing alga by Horikoshi et al. (1981). This particular species is not generally available from Culture Collections. Efforts to obtain a stock culture from the authors were not successful inasmuch as its use is restricted.

The types of batch growth experiments performed during this study are summarized below:

Organism	shake flask	mechanically	Air-lift fermentor
		agitated fermentator	
<u>Rhizopus arrhizus</u>	x	x	x
<u>Streptomyces levoris</u>	x	x	x
<u>Saccharomyces cerervisiae</u>	x	x	
<u>Chlorella vulgaris</u>	x		

Shake flask studies refer to growth experiments in Ehrlenmeyer flasks ranging in volume from 250 mL to 4.0 L. The contents were sterilized by autoclaving at 121°C. The fermentor, tanks, air filters, and feed lines were sterilized by direct steam injection. The liquid media were usually sterilized separately by autoclaving.

The inoculum consisted of 10% (volume basis) freshly grown cells. The progress of growth was followed by Optical Density (Chlorella, Saccharomyces) and dry cell mass concentration (all four species). Additional analysis include determinations of the glucose concentration (Chlorella and fermentor studies) and the pH (fermentor studies) during the time course.

In the mechanically agitated fermentor (Marubisi), heavy wall growth of Streptomyces levoris and Rhizopus arrhizus was observable after short fermentation times. For this reason, the determination of the biomass concentration was not always reliable. This wall growth did not occur in the air-lift fermentor. Therefore, data generated from shake flasks and the air-lift fermentor were judged more reliable. In case of shake flasks, a number of vessels were inoculated simultaneously and

the entire content was used to determine the biomass yield.

The fermentor contents were routinely examined for any evidence of contamination under a compound microscope. Due to standard microbiological practice, contamination was seldom a problem. Occasionally a yeast, tentatively identified as Rhodotorula, was noticed in Chlorella cultures. A few samples of Saccharomyces and Streptomyces became contaminated by an unknown fungus growing on the surface of the biomass after 10 days of storage at 4°C. All cultures showing any evidence of contamination were discarded for use in any of the growth or bioadsorption studies.

3.4 Continuous Cultivation Experiments

Due to the mycelial growth habits of the two principal uranium adsorbing microorganisms, Streptomyces levoris and Rhizopus arrhizus, attempts of chemostat-type continuous cultivations were unsuccessful. The exit lines from the fermentor became rapidly plugged by mycelial clumps, which prevented operating the fermentors in a truly continuous manner. Furthermore, biomass tended to deposit on baffles and around the agitator shaft in the Marubishi fermentor resulting in extensive wall growth and poor cell harvest from the fermentor effluent. For these reasons, a continuously fed-batch operation was adopted as an alternative method.

The term "fed-batch culture" was introduced by Yoshida et al. (1973) to refer to a batch culture in which the nutrient solution is fed continuously to the fermentor, resulting in a variable volume culture system. If a portion of the culture is withdrawn at specified time intervals, the system becomes a "repeated fed-batch culture", which can be maintained indefinitely. The operational characteristics is not unlike the draw and fill waste treatment systems used for pollution control.

As in case of chemostats, fed batch culture is a means of achieving substrate limited growth. Often, fed batch operation increases process efficiency. Yoshida et al. (1973), for example, has reported that the growth yield of Candida tropicalis increased almost two-fold (in comparison to batch culture) when grown in a fed batch manner. It has been shown (Pirt, 1971) that the specific growth rate is dependent on the hydraulic factors such as the influent flow rate and the fermentor volume. The flow rate may be kept constant to study the specific growth rate on cell physiology (Bainbridge et al., 1971) or varied according to a predetermined manner to maintain a constant specific growth rate (Yamane et al., 1981).

The kinetic analysis of fed-batch cultures is straight forward. It has been shown (Pirt, 1971), that constant feeding of a growth limiting substrate tends to give a constant biomass concentrations, provided the flow rate satisfies the following inequality:

$$\frac{F}{V_0 + Ft} \leq \mu_{\max} \quad (3.4.1)$$

where

F = flow rate of feed solution

V_0 = initial volume

$\mu_{\max}$ = maximum specific growth rate

In this case, the "instantaneous" specific growth ($\frac{1}{\sigma} \frac{d\sigma}{dt}$) rate of the culture is given by:

$$\frac{1}{\sigma} \frac{d\sigma}{dt} = \frac{F(t)}{V_0 + \int_0^t F(t) dt} \quad (3.4.2)$$

where:

σ = biomass concentration

$F(t)$ = flow rate (may be time dependent)

If the flow rate is held constant, Equation (3.4.2) reduces to the Equation (3.3.1). Alternatively, the flow rate may be time dependent to give some constant specific growth rate, μ . It can be easily shown, that in this case the time dependence of the flow rate is as follows:

$$F(t) = \frac{\mu V_0}{(1 - \mu t)} \quad (3.4.3)$$

where:

μ = the desired specific growth rate

V_0 = initial volume

t = time

In practice, fed batch operations are particularly useful for maintaining low specific growth rates. This is self evident from Equation (3.4.3). The initial volume and the time cycle between draw and fill must be carefully manipulated to achieve this goal.

In this study, fed batch culturing of S. levoris and R. arrhizus were performed in the air lift fermentor. Analytical methods included periodic determinations of the biomass concentration, the limiting substrate (glucose) concentration; and the pH.

4.0 URANIUM BIOABSORPTION STUDIES

4.1 Analytical Procedures

Two analytical methods were used in this study to determine U(VI) in aqueous solution. The first method relied on neutron activation analysis whereby a measured volume of aqueous solution containing U(VI) (natural isotopic abundance) is dewatered and is placed in a flux of thermal neutrons for a specified time. The U_{235} isotope undergoes fission which produces a chain of radionuclides which decay via delayed neutron emission. Delayed neutron counting at a certain energy is used to evaluate the U_{235} concentration in the sample and the total uranium content. Under circumstances where the uranium in solution is depleted, a variation of the above method is employed. In this case the activation method involves thermal neutron irradiation of U_{238} to U_{239} which decays rapidly to Np_{239} . The neptunium isotope decays via β^- and γ with 75 keV γ emission being measured and related to uranium content. Samples analysed by neutron activation were sent to Nuclear Activation Services Limited, Hamilton, Ontario. The detection limit claimed by N.A.S. was 0.10 mg/L or .1 ppm when a 1 mL sample is used.

A second method of analyses for U(VI) was developed "in house" especially for this project. This method exploited the rather new area of ion chromatography as applied to quantitative analysis of transition metals. A Dionex Ion Chromatograph Model 2010i equipped with an Ionpac[®] Membrane Reactor was employed for analysis. The analytical method has been described in principle by Dionex Corp., however considerable experimental work was required to obtain a reliable analytical procedure applicable to this project. The ion chromatographic procedure for determination of U(VI) as UO_2^{2+} is performed using a separator column supplied by Dionex Corp. This column is made with a

pellicular resin utilizing a latex-agglomerated surface-sulfonated polystyrene-divinylbenzene ion exchange material. This type of exchanger possesses the favourable mass transfer characteristic required for ion chromatography applications. The capacity is approximately $0.020 \text{ mequiv g}^{-1}$ of starting copolymer.

Following ion exchange separation using a solution of $(\text{NH}_4)_2\text{SO}_4/\text{H}_2\text{SO}_4$ as eluent, the eluted UO_2^{2+} is subjected to post column derivatization employing the membrane reactor. The post column reagent was (4-(2-pyridylazo) resorcinol) with the resulting uranyl complex being detected at 520 nm using a micro flow cell installed in a Cary 19 UV-VIS spectrophotometer. A photograph of the analytical equipment is shown in Figure 4.1.1.

A number of factors should be noted with respect to the determination of U(VI) by ion chromatography. At the U(VI) concentrations encountered in the present work (1 to 100 mg/L) dilution of samples must be done in such a way so as to match the sample matrix to the eluent. The optimal eluent concentration was found to be 0.02 M $(\text{NH}_4)_2\text{SO}_4$ and 0.20 M H_2SO_4 at a flow rate of 1 mL/min. The complexing agent concentration was $1 \times 10^{-3} \text{ M}$ dissolved in a solution of 3M $\text{NH}_4 \text{ COOH}$ and 1 M CH_3COOH . The resulting flow rate of reagent through the fiber membrane of the Ionpac® reactor was 0.5 mL/min. The detection limit for this method appears to be 0.005 mg/L or 5 ppb when using a 100 microlitre injection volume.

Concentrations of other metals were determined by standard atomic absorption analytical techniques.



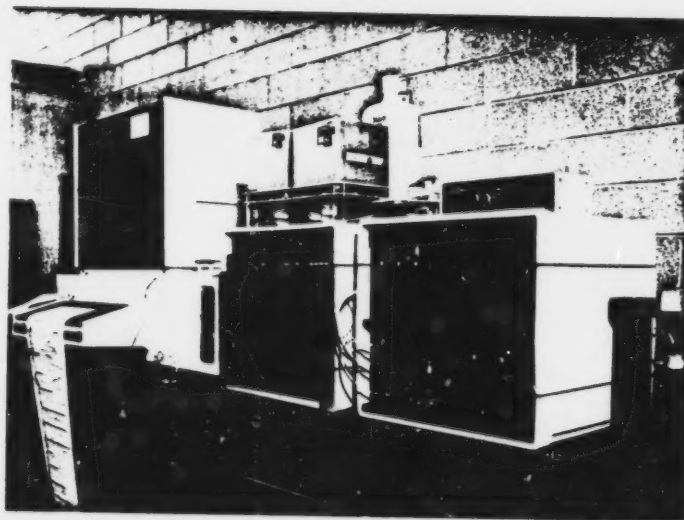


Figure 4.1.1 Inhouse Uranium Analysis Apparatus

4.2 Adsorption Studies - Experimental Procedure

4.2.1 General

Uranium bioadsorption studies were performed in 250 mL Ehrlenmeyer flasks on a Burrell Wrist-Action shaker operated at 300 rpm at 7 degrees angular displacement. Experiments were carried out in duplicate at 3 temperatures: 4°C, 20°C, and 35°C; various pH levels ranging from 2.0 to 5.0; and with both viable and thermally inactivated cells. Several special tests to determine the effects of sulphate and contaminant metals on the uranium loading, as well as tests to determine the maximum uranium concentration were also done with flask and shaker studies.

4.2.2 Experimental Procedures

An outline of the experimental procedure and sampling schedules is provided.

i) Procedure

Weigh out two 0.500 gram samples of both the sterilized and live culture into labelled weighing vessels.

Measure 100 mL of uranium solution into each flask.

Take a 1.0 mL sample of the stock uranium solution.

Set up the two millipore filter units with fresh filters.

Run the four samples and control solution for two hours in the shaker, taking samples according to the following schedule and procedures.



Samples were taken at varying intervals according to the schedule given in Table 4.2.1. This staggering of the sampling allowed for the most efficient use of laboratory equipment and personnel.

ii) Start-up

Stop shaker

Attach flask with uranium solution to shaker

Add biomass to flask if required

Restart shaker

iii) Sampling

Stop shaker

Withdraw 5 mL of solution with the 10 mL pipette, and transfer this solution into a filter unit.

(Repeat for second sample if required).

Start shaker

Take 1.0 mL sample of filtered solution with 1 mL pipette, and place sample in plastic vial.

Rinse pipettes with distilled-deionized water.

Clean and rinse filter units with distilled-deionized water, collecting the used filter paper for later sterilization



Table 4.2.1

SAMPLING SCHEDULE FOR SHAKER FLASK BIOADSORPTION STUDIES

<u>Time On (min)</u>	<u>Sample</u>	<u>Time Sampled (min)</u>
0	control	15
5	live culture 1	20
5	dead culture 1	20
10	live culture 2	25
10	dead culture 2	25
15	control	30
20	live culture 1	35
20	dead culture 1	35
25	live culture 2	40
25	dead culture 2	40
30	control	60
35	live culture 1	65
35	dead culture 1	65
40	live culture 2	70
40	dead culture 2	70
60	control	120
65	live culture 1	125
65	dead culture 1	125
70	live culture 2	130
70	dead culture 2	130

Dry and assemble filter units with new filter paper.

iv) Shutdown

Stop shaker

Detach flask from shaker

Start shaker

Take final sample from detached flask

Sterilize solutions containing live cultures before disposal

Clean up equipment and work area.

Package and send samples to an independent laboratory for analysis of uranium content in the solution or submit sampler for "in house" uranium determination.

4.2.3 Testing Program

The overall testing program for bioadsorption studies is summarized in Table 4.2.2. As noted previously where practical all experiments were carried out in duplicate. All standard experiments were performed with process liquor as test solution. This solution was obtained from Denison Mines and was identified as mine water from underground leaching operations. This solution varied in uranium concentration both on an as received basis and when pH was adjusted. Test solutions were analyzed to obtain standard error measurements for the analytical technique.

For tests in which sulphate and metal contaminants were

Table 4.2.2

URANIUM BIOADSORPTION - VARIABLES TESTED

BIOMASS	pH			Contaminant Effects						
	5.0			3.5	2.0					
	4°C	20°C	35°C	20°C	20°C	SO ₄	Fe ³⁺	Ni	Cu	Zn
<u>Rhizopus arrhizus</u> inactivated viable	x x	x x	x x	x	x x		x			
<u>Streptomyces levoris</u> inactivated viable	x x	x x	x x	x	x x	x x	x x	x x		x
Mixed Culture inactivated viable	x x	x x	x x	x	x x		x			
<u>Chlorella vulgaris</u> inactivated viable	x x	x x	x x							
<u>Saccharomyces cerevisiae</u> inactivated viable	x x	x x	x x							
<u>Rhizopus oligosporus</u> inactivated viable				x	x x					
<u>Chaetomium cellulolyticum</u> inactivated viable										

introduced, a synthetic uranyl nitrate test solution was employed to better define contaminant effects. This latter aspect was a modification in scope which was instituted to try to explain the wide variation in results. Given the success of the preliminary desorption studies, the level of effort was decreased in that area and transferred to the testing of contaminant effects.

4.3 Equilibrium Studies

Equilibrium studies were conducted in the same fashion as the loading studies excepting a known concentration of biomass was contacted with 100 mL of process solution at 20°C and shaken for 90 minutes. Samples were collected and another 100 mL added and this procedure repeated until a total of 400 mL of process solution was contacted with the biomass. This data was collected in order that an estimate of the maximum loading capacity for each biomass could be obtained. Selected studies with young versus aged cells were also conducted.

Testing was done as shown on Table 4.3.1 and included tests at pH 3.5, 4 and 5 on inactivated cells. Streptomyces was also tested with viable cells at pH 5.0. Uranium concentrations varied from high to low depending upon the source strength of the process liquor.

4.4 Batch Desorption Studies

The batch desorption studies were used as a screening method to assess the preferred desorbing solutions. For these studies biomasses were loaded at pH 3.9 or 3.5 and 20°C in the standard shaker flask with shaker times of 90 minutes. This was followed by desorption with 4 solutions, .02 N H₂SO₄ (pH = 2.05), 0.1 M (NH₄)₂SO₄ (pH = 5.3), 0.02 M EDTA (disodium salt, pH = 4.7) and 0.1 M NaHCO₃ (pH = 8.2). For these tests, inactivated Rhizopus

Table 4.3.1

EQUILIBRIUM STUDIES VARIABLES TESTED

	pH		
	3.5	4.0	5.0
<u>Rhizopus arrhizus</u> inactivated	x	x	x
<u>Streptomyces levoris</u> inactivated	x	x	x
viable			x
Mixed Culture inactivated	x	x	x
<u>Chlorella vulgaris</u> inactivated	x	x	x
<u>Saccharomyces cerevisiae</u>		x	

arrhizus, Streptomyces levoris and mixed culture were utilized.

4.5 Column Absorption/Desorption Studies

Procedure

The columns used in this study were prepared with glass wool packing, then demineralized sand acting as a lower barrier for the cells, and in some cases a glass wool top plug to prevent the flotation of cells above the bed.

The columns were then run with distilled, deionized water to further pack the columns, as well as to give the experimenters a chance to observe the column characteristics before running the uranyl ion solution.

The column flow rate was then adjusted with distilled deionized water to the flow required for the specific test.

Next the constant head distribution system was charged with 3.0 L of uranium solution for each column to be run.

The column was then fed with the uranium solution, and sampling was carried out as given in Table 4.5.1.

The samples were taken by collecting the final 5 mL in each test period, and pipetting out a 1.0 mL sample into a plastic sample vial. This 5 mL sample collection was also used to ensure that the columns were running at the desired 5.0 mL/min flow rate.

After 3000 mL of uranium solution had been processed, two 50 mL aliquots of distilled deionized water were then run through the column, with 1.0 mL of sample being taken from each 50 mL collected from the column during this washout step.

Table 4.5.1

COLUMN SAMPLING SCHEDULE

<u>Solution Passed Through Column (mL)</u>		<u>Sample Time (min)</u>
UO ₂ ²⁺	100	20
	200	40
	300	60
	400	80
	500	100
	600	120
	700	140
	800	160
	900	180
	1000	200
	1200	240
	1400	280
	1600	320
	1800	360
	2000	400
	2200	440
	2400	480
	2600	520
	2800	560
	3000	600
Pure Water	25	n/a
	25	n/a
Na ₂ CO ₃	10	n/a
	20	n/a
	50	n/a
	100	n/a

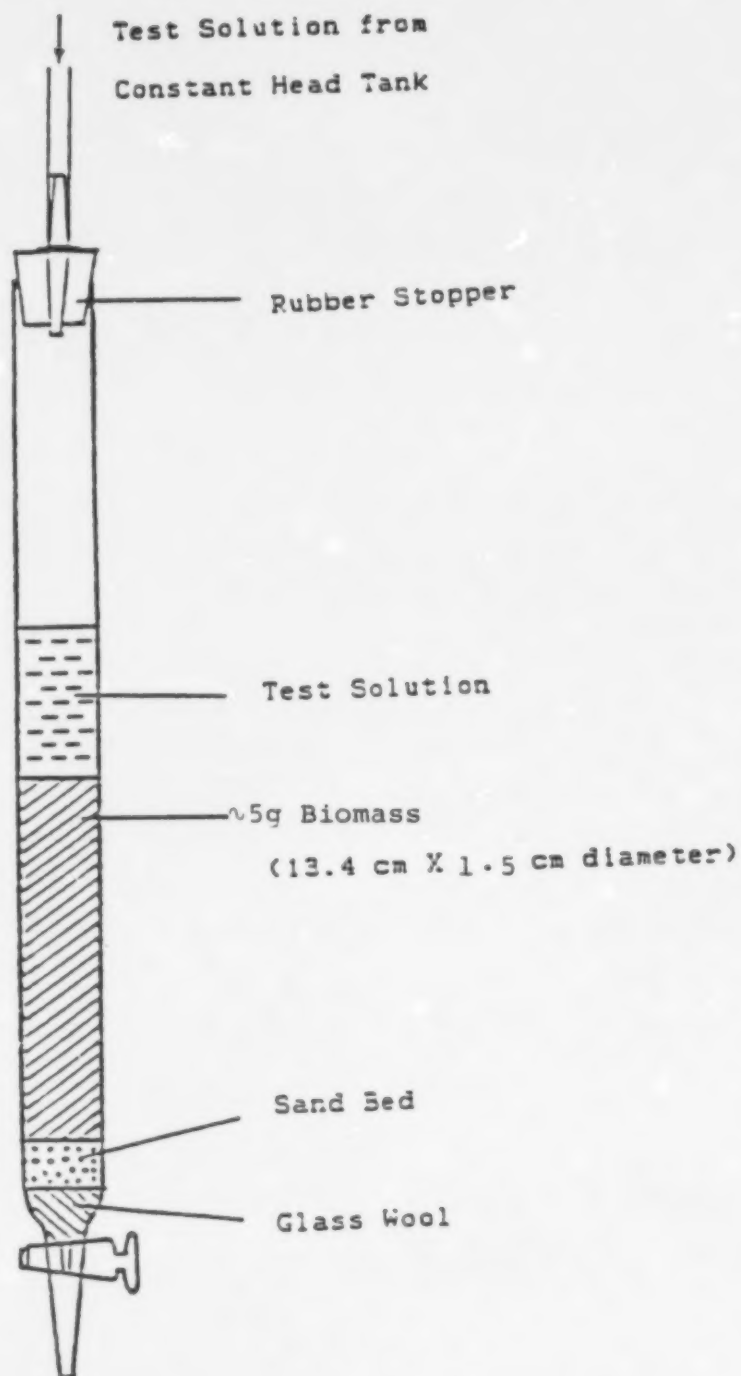


FIGURE 4.5.1 APPARATUS FOR COLUMN EXPERIMENTS

The column was then run with bicarbonate solution to deadsorbed the uranium from the cell walls. As in the water rinse, here the 1.0 mL sample was taken from the total volume of sample collected during each bicarbonate aliquot addition.

Finally the column was run with water in a final rinse step, and the samples were sent for analysis.

4.5.2 Testing Program

Screening tests were conducted with Rhizopus to determine acceptable flow through characteristics and loading capacities. This was followed by three cycles of adsorption/desorption with the three biomasses. Loading was conducted with the process liquor at pH=3.5 and desorption was conducted with 0.1 M NaHCO₃, pH=8.2.

Three biomasses were selected for column loading and desorption testing, Rhizopus arrhizus, Streptomyces levoris and mixed culture. Only the mixed culture was successfully immobilized and achieved acceptable column flow through characteristics, however, results for immobilized Rhizopus and Streptomyces are also presented.

Biomass immobilization has been proven to be an economically attractive technology in the biochemical industry. Immobilization allows the conservation of the biomass, enables convenient, easy recovery and often enhances stability and structural integrity. The advances in the techniques of whole cell immobilization have been reviewed periodically (Durand and Navarro, 1978; Wiseman, 1980; Vojtisek and Jirku, 1983). Common techniques of cell immobilization include attachment to inert supports, physical entrapment in polymeric materials, chemical



cross-linking, and physical aggregation. Of the above methods, chemical cross-linking of whole cells appeared to be the most effective means of maintaining high adsorption capacity coupled with low mass transfer resistance for uranium bioadsorption.

Based on the published reviews and research by Hirano et al. (1977) and Tanaka et al. (1982), glutaraldehyde was selected as an economically attractive cross-linking reagent. The reagent was tested at concentrations of 0.5, 1.0, 2.5, 4.0 and 5%, at pH values of 2.0, 6.0, and 9.0. The reaction time was varied from 2 hours to 24 hours. Immobilization tests were performed at 4°C, 20°C and 80°C.

High glutaraldehyde concentrations (1% and above) produced good cross-linking, but substantial degradation and charring of the biomass occurred. After a considerable research effort, the following procedure was developed.

Ten grams (wet weight) of biomass was mixed with 5 mL of 95% ethanol and 10 mL of 0.5% glutaraldehyde in a 100 mL beaker and let stand for 2 hours at 4°C. After 2 hours, 20 mL of fresh glutaraldehyde was added and the contents were stirred with a magnetic stirrer for 10 minutes. After stirring, the biomass was let stand another 2 hours or longer until a granular texture developed. Then, the liquid was filtered off, the biomass was gently washed with water, and used to pack the columns.

5.0 RESULTS - GROWTH STUDIES AND BIOMASS PRODUCTION

5.1 Rhizopus arrhizus

Typical batch growth curve for Rhizopus arrhizus is shown on Figure 5.1.1. This microorganism was one of the easiest to cultivate and harvest. In the Marubishi fermentor and in shake flasks, the fungus grew in clumps or pellets reaching approximately 5 cm in length and 0.5 cm in thickness. Inoculum homogenized in a sterilized Waring Blender prior to inoculation invariably gave rise to the re-formation of the pellets after 5-10 hours fermentation time. This pellet formation, however resulted in very rapid harvesting by filtration. In fact, much of the mycelia could have been recovered "by hand". In the air lift fermentor, pellet formation did not occur and the mycelial suspension remained homogeneous. The hyphae became somewhat discoloured after excessive fermentation time although evidence of sporulation (Lawler and Weber, 1977) was not observable by microscopic examination. The growth cycle was normally completed in 48 hours. During the growth phase, the pH declined to pH = 3.2-3.5 in batch fermentations.

5.2 Streptomyces levoris

A growth curve of Streptomyces levoris is shown on Figure 5.2.1. After a short lag period, this microorganism grew at a somewhat higher specific growth rate than Rhizopus arrhizus. In the Marubishi fermentor and shake flasks, Streptomyces levoris had a particularly strong tendency to deposit on solid surfaces as filamentous chains of cells embedded in a mucoid presumably polysaccharide, excretion product. Suspended cells formed loose, spherical pellets of approximately 3-5 mm diameter. Cells in pellets and in wall growth were morphologically identical. The characteristic, "earthy" odour of Streptomyces was very evident

FIGURE 5.1.1 GROWTH CURVE FOR Rhizopus arrhizus
IN THE AIR LIFT FERMENTOR

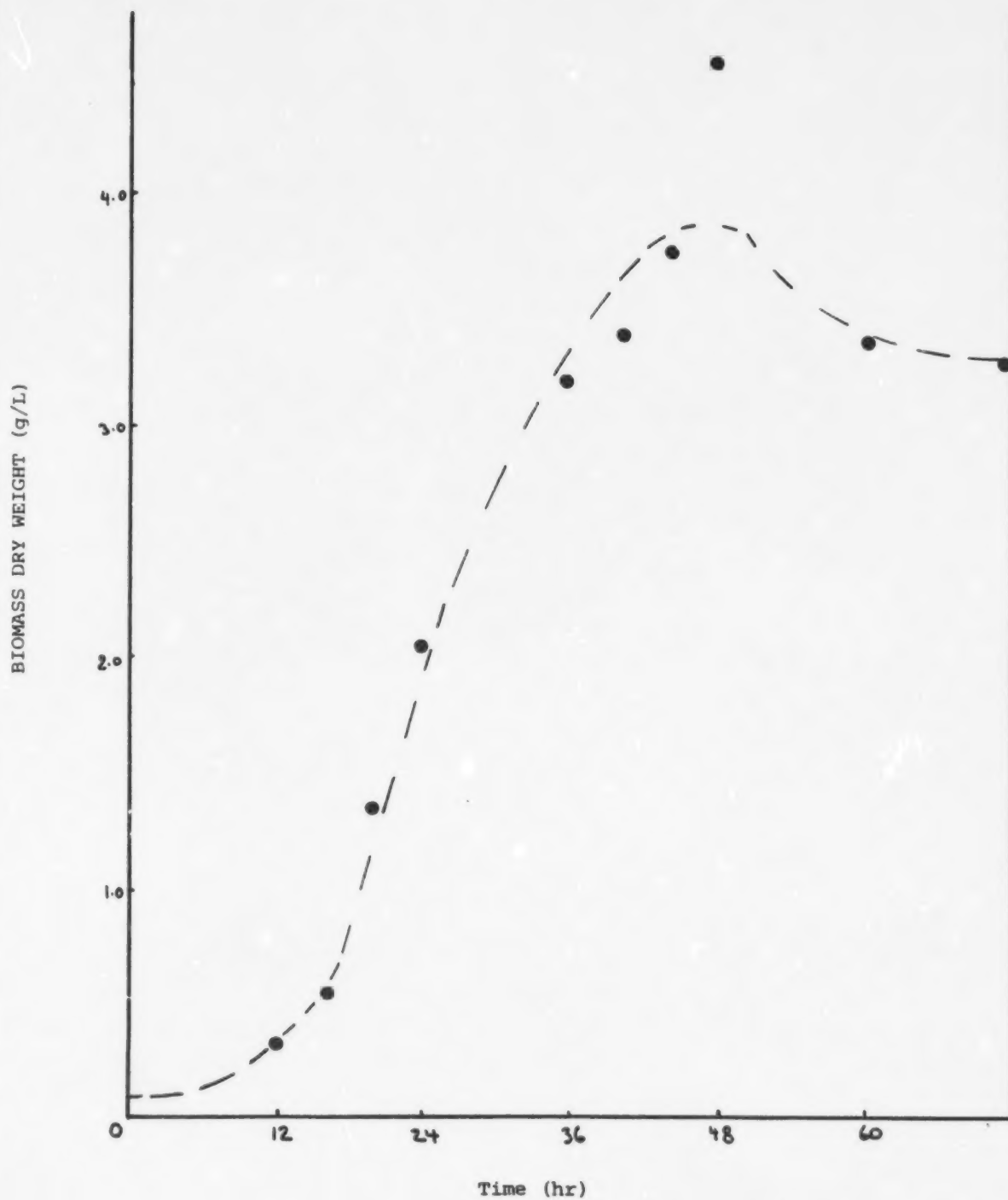
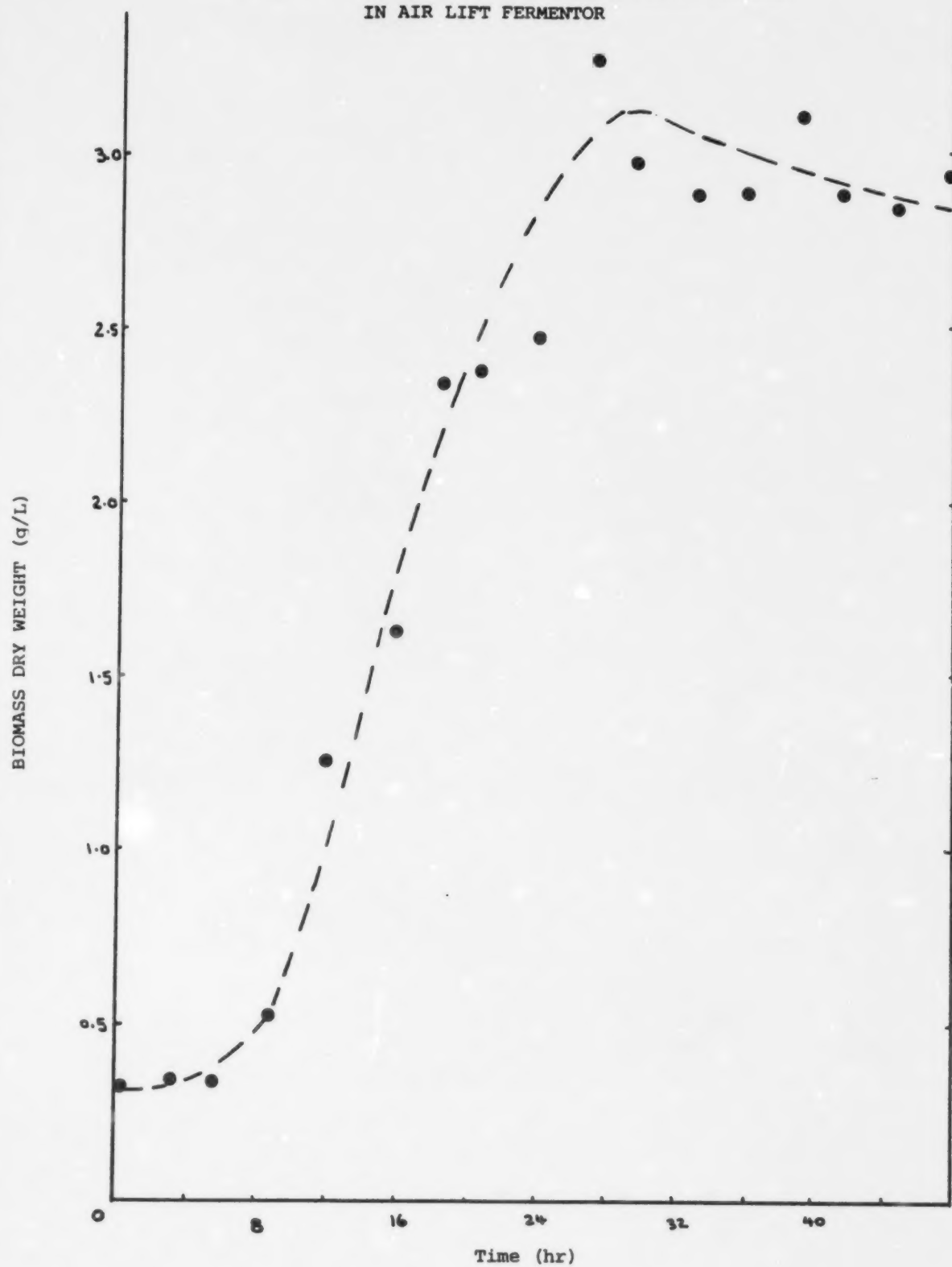


FIGURE 5.2.1 GROWTH CURVE FOR Streptomyces levoris
IN AIR LIFT FERMENTOR



during the fermentations. Some foaming in cultures in their late logarithmic phase of growth occurred that required the addition of an antifoam (silicone oil) reagent. In contrast to Rhizopus arrhizus the pH of the broth gradually increased during the reaching a final value of pH = 8.2 - 8.5. The fermentation was normally completed in 36-48 hours.

The growth of Streptomyces levoris required a rather complex medium. Growth experiments with reduced tryptone content were performed to derive a simpler growth medium. However, any reduction in tryptone content adversely affected the growth yield (See Appendix B-1).

5.3 Saccharomyces cerevisiae

Of the four microorganisms studies, Saccharomyces cerevisiae possessed the highest growth rate. Typical growth curve of Saccharomyces cerevisiae is shown on Figure 5.3.1. Since the yeast culture in the fermentor fluid remained homogeneous the extent of growth was determined by both Optical Density measurements and dry weight determinations. Usually the fermentation was completed in 24-30 hours. During this fermentation time, the pH declined to pH = 4.2 to pH = 4.5. Although Saccharomyces is a facultative organism, it has been grown aerobically only in this study.

5.4 Chlorella vulgaris

Usually, Chlorella was cultivated in 4L culture flasks containing 2 L medium. To produce sufficient cell mass for the bioadsorption studies, the alga was grown heterotrophically. A representative growth curve is shown on Figure 5.4.1. The growth period for the alga was 5-6 days even in the heterotrophic medium. It has been shown previously (Horikoshi, 1981) that the

FIGURE 5.3.1 GROWTH CURVE FOR Sacchromyces cerevisiae
IN THE MARUBISHI FERMENTOR

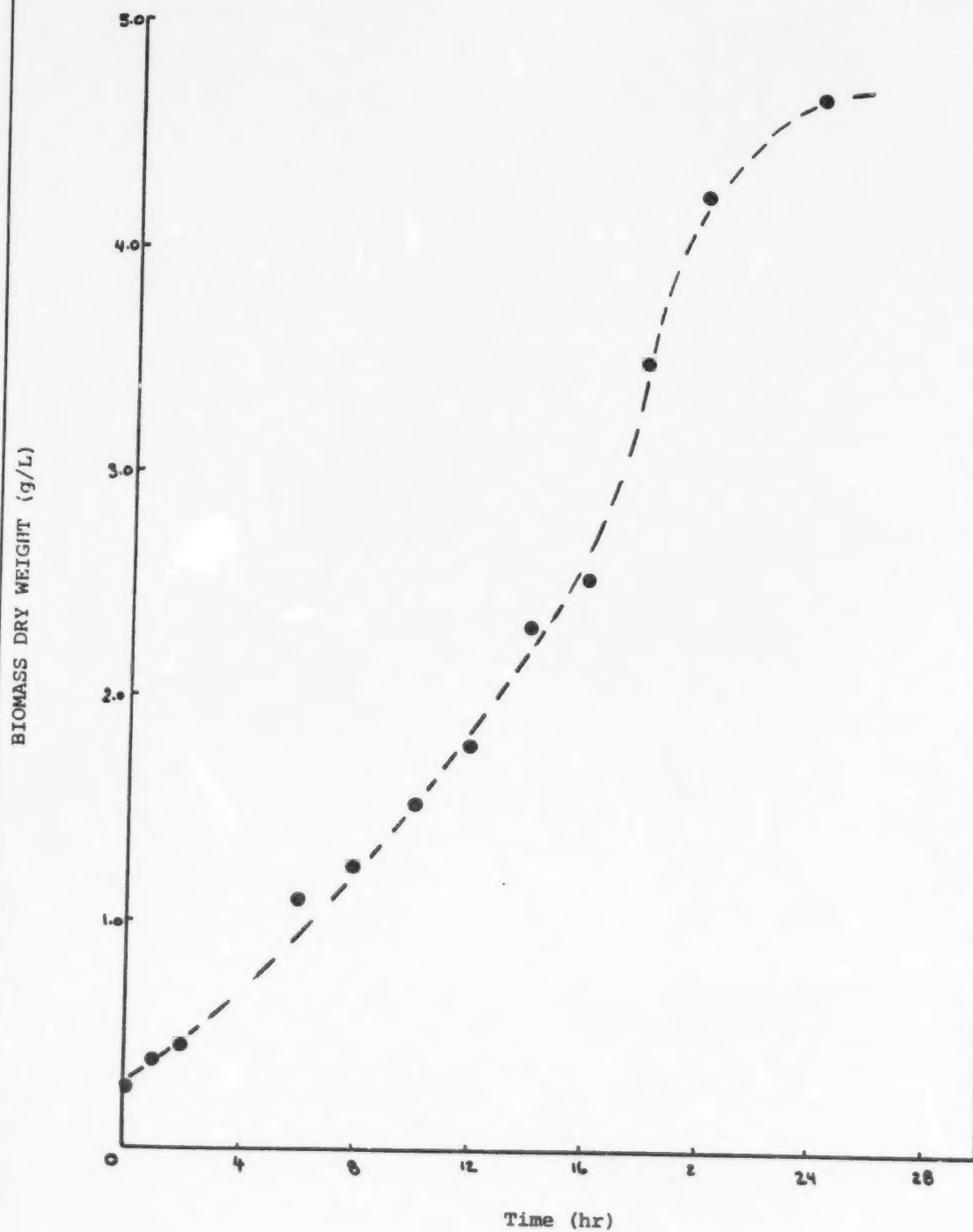
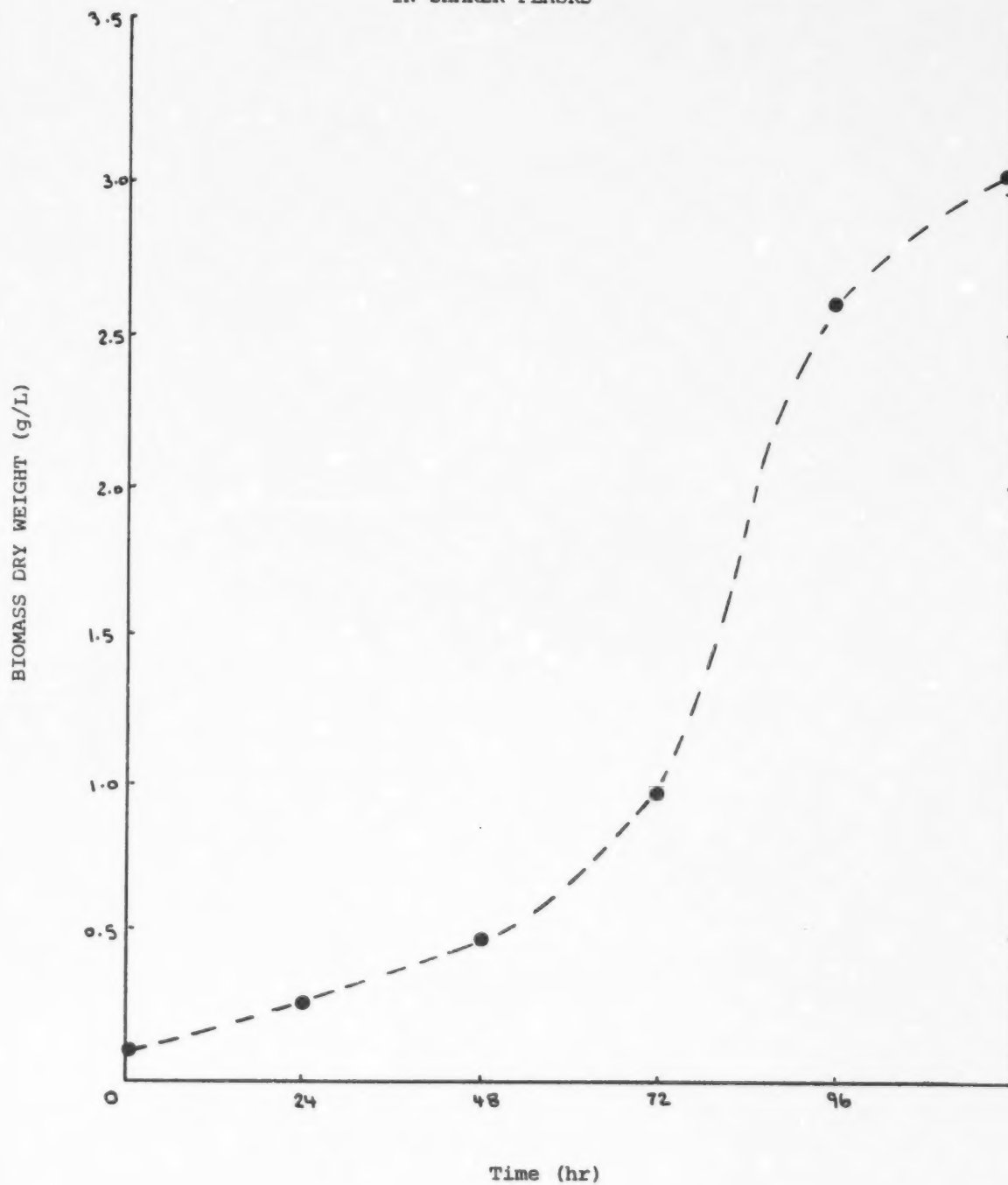


FIGURE 5.4.1 GROWTH CURVE FOR Chlorella vulgaris
IN SHAKER FLASKS



uranium bioadsorption in heterotrophically, mixotrophically, and autotrophically grown alga is identical. The algal cell mass was harvested by centrifugation.

5.5 Discussion

Typical examples of the experimental data and sample calculations for the fed-batch studies are given in Appendix B.1. The flow rate of medium into the vessels was controlled manually to maintain an apparent specific growth rate of $\mu = 0.01 \text{ hr}^{-1}$ which is approximately 1/20 of the maximum specific growth rate. As indicated by the constant values of the biomass and glucose concentrations, stationary states are achieved after 2 repeated fed-batch cycles.

The growth characteristics of the microorganisms are summarized in Table 5.5.1. The data represent the averages of at least three independent growth studies. The maximum specific growth rates (μ_{max}) were calculated from log-transformed biomass concentration data. The enhanced growth rates in the air-sparged vessels reflect better mass transfer characteristics. Growth yields, i.e. g biomass produced from 1 g growth limiting substrate, were based on glucose as the limiting nutrient. The growth yields of Rhizopus arrhizus and the Saccharomyces cerevisiae are well within the range of microbial growth under aerobic conditions. Somewhat lower than typical aerobic growth yield was observed in case of Streptomyces levoris cultures. It is worthwhile to interject at this point that this organism was previously considered an Actinomyces implying an anaerobic growth habit, hence, lower biomass yield. The very low growth yield of the Chlorella reflects the primarily autotrophic nature of algae.



Table 5.5.1

GROWTH CHARACTERISTICS OF MICROORGANISMS

<u>Microorganism</u>	Maximum Specific	Growth Yield (g/g)	
	<u>Growth Rate (hr⁻¹)</u>	<u>Batch Culture</u>	<u>Fed-Batch Culture</u>
<u>Streptomyces</u> <u>levoris</u>	0.11 (shake flask) 0.22 (air lift)	0.31	0.34
<u>Rhizopus</u> <u>arrhizus</u>	0.096 (shake flask) 0.20 (air lift)	0.44	0.48
<u>Saccharomyces</u> <u>cerevisiae</u>	0.198 (shake flask) 0.237 (Marubishi)	0.42	-
<u>Chlorella</u> <u>vulgaris</u>	0.033 (shake flask)	0.12	

6.0 BIOADSORPTION STUDY RESULTS

6.1 Chemical Characteristics of Process Liquor

As noted in Section 4.0, process liquor was supplied by Denison Mines Limited. This solution was minewater from the underground leaching operations. The minewater was used as received at pH 2.0 and at several intermediate pH values between 2.0 and 8.0 after adjustment with sodium hydroxide.

The uranium contents as well as other key characteristics for each pH level are shown on Table 6.1.1. Also shown on Table 6.1.1 is a typical mine water analysis. In general, the process solutions tested were higher in acidity and uranium content that is typical of the Denison mine waters.

At pH of 5, the uranium content was highly variable as minor changes in pH at this level resulted in marked uranium concentration differences. For testing purposes, the process liquor at pH 2.0 was pH adjusted by sodium hydroxide filtered and readjusted before commencement of bioadsorption studies to ensure test solutions were at their proper pH.

6.2 Uranium Bioadsorption Studies

The experimental results pertaining to this section are tabulated in Appendix B-2. In this section, the effect of the physiological and physical parameters on the kinetics of the bioadsorption are discussed. The physiological parameters include biomass type, viability (vs. thermal inactivation) and physiological age. The physical parameters comprise the temperature effect and the pH on the uranium bioadsorption phenomenon. In additional experiments, the effect of the ions, notably sulphate and metal ions on uranium was investigated in

Table 6.1.1

COMPARISON OF PROCESS LIQUOR
TEST SOLUTIONS

	TYPICAL MINE* WATER	AS REC'D pH=2	PROCESS LIQUOR			AS ADJUSTED TO pH	
			3	3.5	4.0		
pH	2.7	2.0	3	3.5	4.0	5.0	8.0
Uranium mg/L	127	234.5-244	233	184-256	221	42.6-127.5	<1
Iron mg/L	270	1056	-	13	-	19	-
Zinc mg/L	3.6	12	-	8.3	-	9	-
Copper mg/L	1.0	9	0	2.2	-	1	-
Sulphate mg/L	1300	2543	2543	2543	2543	2543	2543

* Other elevated contaminants include Ca, Mg, Co, Ni, and Al.

synthetic solutions of known composition.

As discussed in Section 2.2, the concentration of dissolved uranium species in contact with biomass tend to reach equilibrium concentrations. In a closed system, this equilibrium concentration is dependent, besides the before mentioned physiological and physical parameters, on the total biomass concentration and the initial uranium concentration in the system. The kinetics of the bioadsorption, i.e. the rate of approach to thermodynamic equilibrium, reflects the transfer of mass from the liquid to the solid surface.

Typical uranium bioadsorption curves from pH adjusted process liquor (initial pH = 5.0) for each of the biomasses (thermally inactivated) at 20°C are shown in Figures 6.2.1 to Figure 6.2.7. The tendency toward equilibrium is a common feature of these curves. It is also evident, that the rate of the adsorption reactions and the finite (equilibrium) distribution of uranium varies from one type of biomass to another. In general, small cells in homogeneous suspensions (Chlorella, Saccharomyces) essentially approach equilibrium within 15 minutes of adsorption time. These are followed by loose bacterial aggregates (Streptomyces), bacterial flocs (mixed culture) and mycelial aggregated (Chaetomium, Rhizopus).

The effect of thermal inactivation on the uranium bioadsorption capacity is shown in Table 6.2.1. The uranium bioadsorptions represent equilibrium values calculated from kinetic data by non-linear parameter estimation (SAS method) with Equation 2.2.18 as the kinetic expression. The parameter of interest is bioadsorption ratio, i.e. the estimated equilibrium bioadsorption capacity ratio of inactivated to viable cells. As shown in Table 6.2.2, the bioadsorption capacity improves somewhat with thermal inactivation in Rhizopus and Streptomyces species, remains

Table 6.2.1

EFFECT OF THERMAL INACTIVATION ON URANIUM BIOADSORPTION CAPACITY
pH 5.0, 20°C

<u>SPECIES</u>	<u>INITIAL URANIUM CONC. (mg/L)</u>	<u>URANIUM BIOADSORPTION (mg/g) (Viable Cells)</u>	<u>URANIUM BIOADSORPTION (mg/g) (Inactivated Cells)</u>	<u>BIOADSORPTION RATIO (Inactivated/viable)</u>
<u>Rhizopus arrhizus</u>	24.86	13.36	15.53	1.16
<u>Streptomyces levoris</u>	33	42.31	50.1	1.18
<u>Mixed Culture</u>	33	31.66	29.38	0.93
<u>Chlorella vulgaris</u>	65.3	28.28	21.18	0.75
<u>Saccharomyces cerevisiae</u>	24.86	15.64	15.3	0.98
<u>Rhizopus oligosporus</u>	65.3	34.66	64.72	1.87
<u>Chaetomium cellulolyticum</u>	65.3	12.1	8.85	0.73

FIGURE 6.2.1 - URANIUM BIOADSORPTION WITH INACTIVATED Rhizopus arrhizus, pH 5.0, 20°C

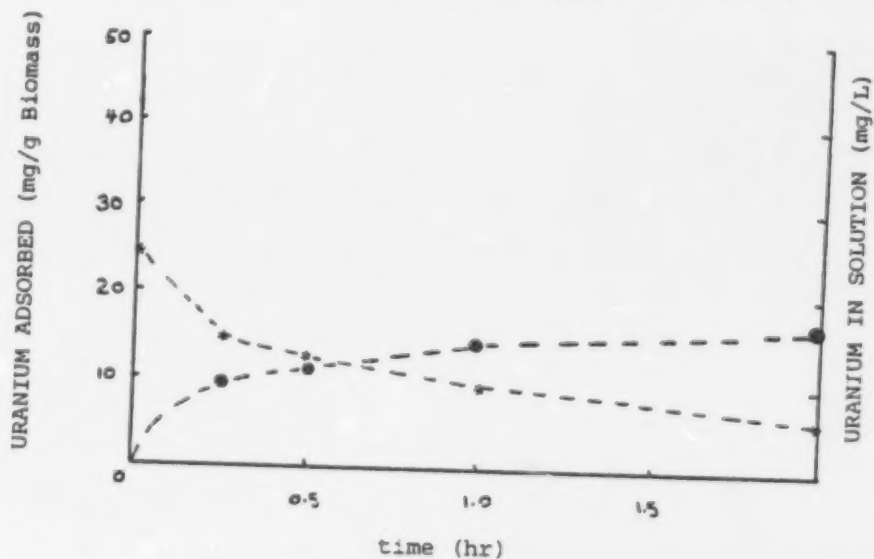


FIGURE 6.2.2 - URANIUM BIOADSORPTION WITH INACTIVATED Saccharomyces cerevisiae, pH 5.0, 20°C

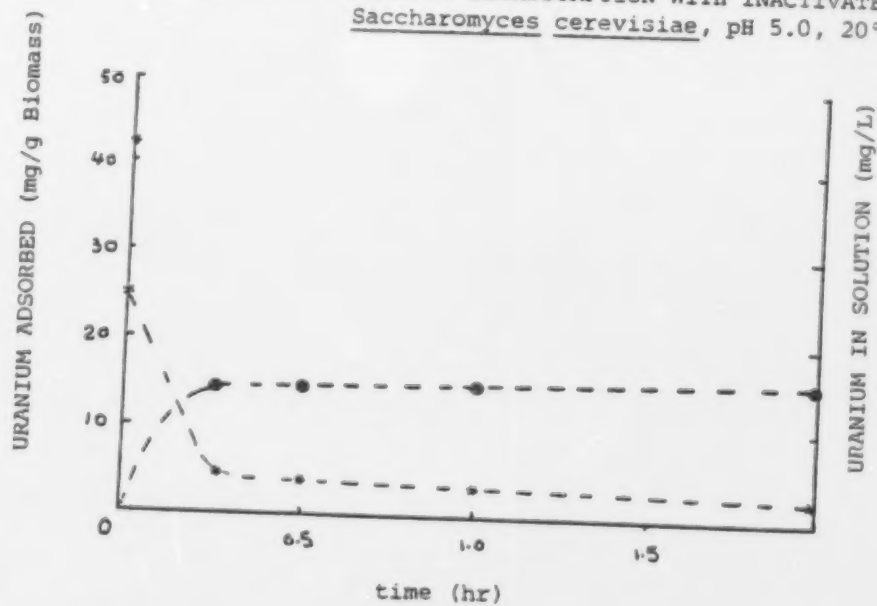


FIGURE 6.2.3 - URANIUM BIOADSORPTION WITH INACTIVATED MIXED CULTURE, pH 5.0, 20°C

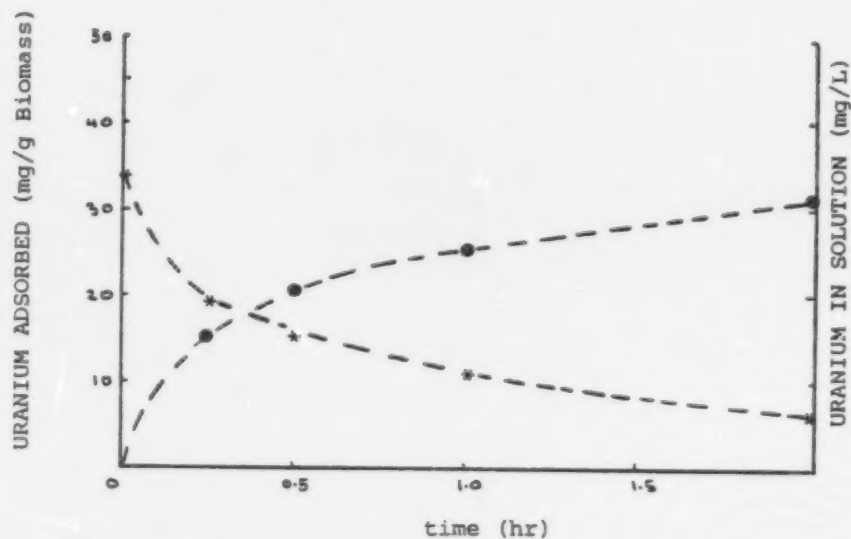


FIGURE 6.2.4 - URANIUM BIOADSORPTION WITH INACTIVATED Streptomyces Levoris, pH 5.0, 20°C

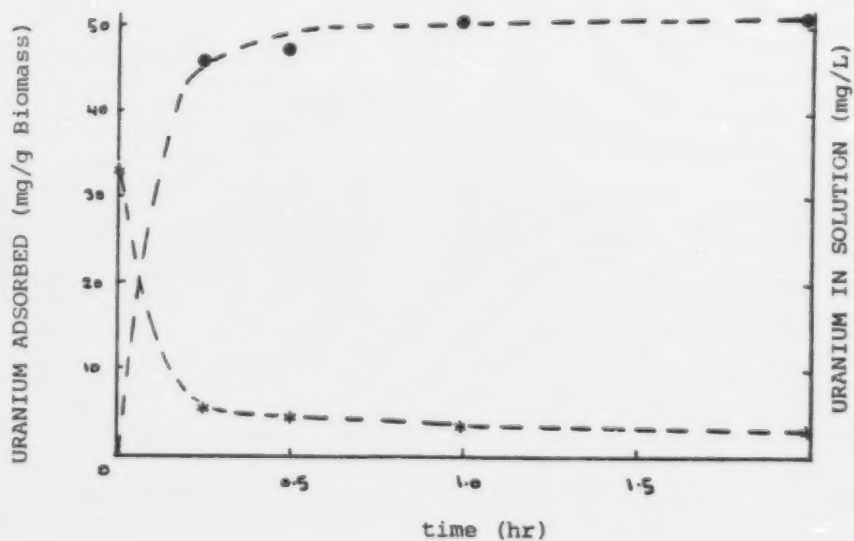


FIGURE 6.2.5 - URANIUM BIOADSORPTION WITH INACTIVATED *Chlorella vulgaris*, pH 5.0, 20°C

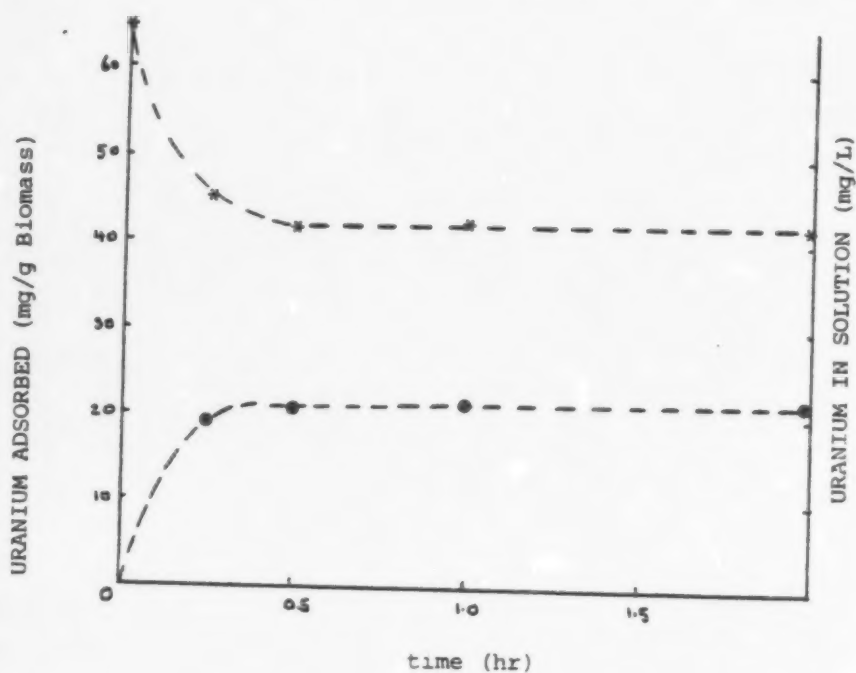


FIGURE 6.2.6 - URANIUM BIOADSORPTION WITH INACTIVATED *Rhizopus oligosporus*, pH 5.0, 20°C

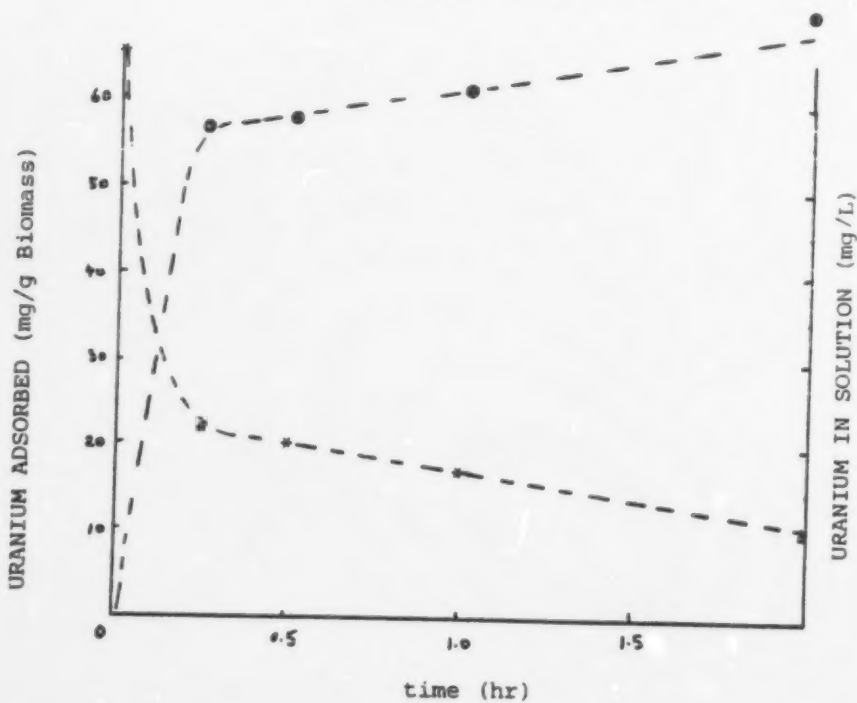
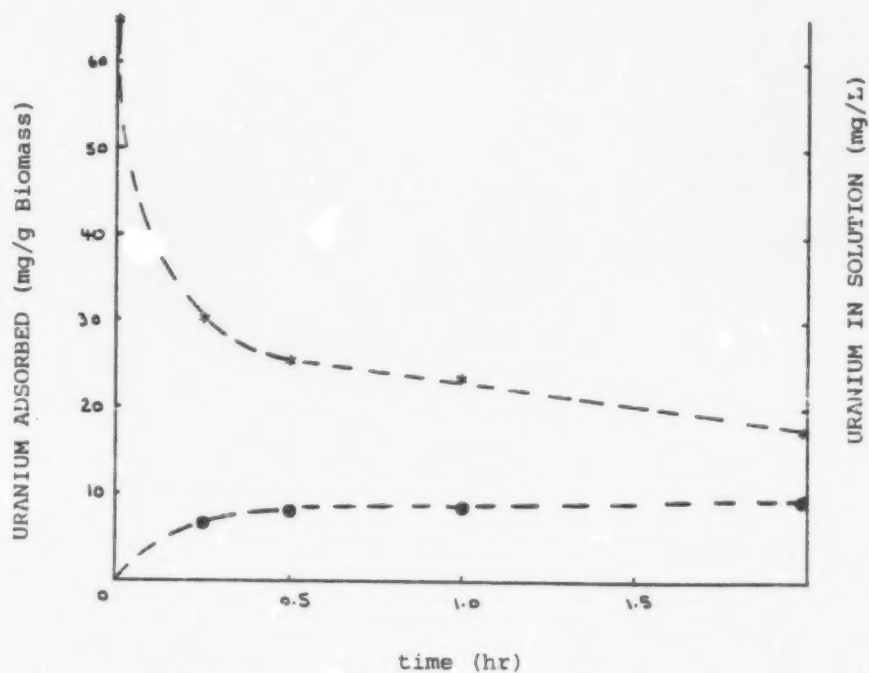


FIGURE 6.2.7 - URANIUM BIOADSORPTION WITH INACTIVATED
Chaetomium cellulolyticum, pH 5.0, 20°C



virtually unchanged in mixed culture and Saccharomyces suspension and declines in case of Chlorella. In most cases, the bioadsorption ratio does not substantially differ from 1.0 (i.e. no effect on equilibrium adsorption capacity). Considerably more significance can be deduced from the effects of inactivation on the empirical bioadsorption reaction rate constant (see Equations 2.2.17 and 2.2.18). These constants are summarized for organisms forming cell aggregates in Table 6.2.2. As shown in this table, the ratio of the reaction rate constants increases by a factor of 2 or more in these cultures when exposed to thermal processes. This reflects probable physical changes in mycelial aggregates and flocs as a result of the heat treatment. An increase in the reaction rate constant implies faster approach to equilibrium, hence higher apparent (non-equilibrium) bioadsorption capacity at a specific experimental time period. The beneficial effect of heat treatment on the bioadsorption capacity of fungi has been noted previously (Galun et al., 1981; 1983). From the above analysis, it appears, that thermal treatment enhances the rate of adsorption resulting from physical modification of cell aggregates, while the equilibrium adsorption capacity remains virtually unchanged. In case of unicellular suspensions, the inactivation had little effect on the uranium adsorption capacity.

Due to different initial uranium concentrations, interspecies comparison of the bioadsorption capacities is not straightforward. Process solutions adjusted to pH = 5, resulted in the precipitation or coprecipitation of the uranyl ion with ferric hydroxide, which gave widely differing dissolved uranium content in spite of careful pH adjustment measures. Furthermore, the dissolved uranium levels changed as the solution aged. Nevertheless, the data base indicates good adsorption capacity in Streptomyces levoris, and Rhizopus oligosporus, and mixed culture systems followed by Chlorella vulgaris and

Table 6.2.2

EFFECT OF THERMAL INACTIVATION
ON EMPIRICAL BIOADSORPTION RATE CONSTANT k

SPECIES	k VIABLE CELLS	k INACTIVATED CELLS	RATIO INACTIVATED/VIABLE
<u>Rhizopus arrhizus</u>	1.83	3.48	1.90
<u>Streptomyces levoris</u>	8.17	10.41	1.27
Mixed Culture	1.31	2.77	2.11
<u>Rhizopus oligosporus</u>	2.92	8.16	2.79

Saccharomyces cerevisiae. The fungus, Rhizopus arrhizus has been reported to be one of the most efficient uranium adsorbing organisms (Tzezos and Volesky, 1981, 1982 1983; Tobin et al. 1984). The poor performance of this organism indicated in Table 6.2.1 was believed to be due to its tendency to clump. In all further studies, these clumps were eliminated by short homogenizing in the Waring blender resulting in considerably better performance.

Studies concerning the effect of physiological age on the bioadsorption capacity were inconclusive. A slight decline (20%) in the bioadsorption capacity Streptomyces levoris grown at low specific growth rates in semi-continuous cultures was noted, while Rhizopus arrhizus was not affected. It appears that the adsorption capacity is dependent on physiological variable controlled by factors other than the specific growth rate such as medium composition (Berthelin and Munier-Lamy, 1983). The study of medium composition on the bioadsorption capacity, however, was beyond the scope of the present work.

The effect of pH on the equilibrium bioadsorption capacity is illustrated for thermally inactivated cells in Table 6.2.3. The data were derived for process liquor, thus, the test solution concentrations reflect dissolved uranium as they were affected by pH adjustment. In addition, bioadsorbing liquids adjusted to pH = 5.0 usually declined to pH values of between pH = 4.0 and pH = 5.0 during the course of bioadsorption experiments. This was attributed to the lack of buffering capacity in the liquid. Considering residual concentrations of dissolved uranium (see Appendix B.2) and equilibrium studies (Section 6.3), the bioadsorption given in Table 6.2.3 was near the maximum adsorption capacity at pH=2, approximately 45-55% of the maximum adsorption capacity at pH=3.5, and 20 to 30% of the maximum at pH=5.0. These observations infer the adverse effect of



Table 6.2.3

COMPARISON OF EQUILIBRIUM BIOADSORPTION CAPACITY X_{eq}
AND EMPIRICAL RATE CONSTANT k

SPECIES INACTIVATED	pH = 5.0 20°C		35°C		pH = 3.5 20°C		pH = 2.0 20°C			
	X _{eq}	k	X _{eq}	k	X _{eq}	k	X _{eq}	k		
<u>Rhizopus arrhizus</u>	24.58	6.58	15.53 (2)	2.38	42.13	3.54	20.9	3.27	16.8	4.19
<u>Streptomyces levoris</u>	14.63	1.96	50.1 (1)	10.41	31.35	5.58	20.81	4.23	36.5	3.22
Mixed Culture	36.2	1.68	29.38 (1)	2.77	50.7	4.87	30.4	10.0	14.83	7.9
<u>Chlorella vulgaris</u>	31.39	6.49	21.18 (3)	9.17	29.4	5.16	-	-	-	-
<u>Saccharomyces cerevisiae</u>	24.33	8.44	15.3 (2)	2.28	36.55	9.74	28.96	5.82	9.88	-
<u>Rhizopus oligosporus</u>	-	-	64.72 (3)	8.16	-	-	39.02	1.27	-	-
<u>Chaetomium cellulolyticum</u>	-	-	8.85	5.87	-	-	12.69	6.91	-	-

Test Solution Conc
mg/L - U

47

33(1)

24.8(2), 65.3(3)

55.4

177

229

decreasing pH values on uranium adsorption capacity. The results are in agreement with data published by Tzesos and Volesky (1981), Crist et al. (1981), and Galum et al. 1981 using other bioadsorbing organisms.

More direct evidence on pH effects has been provided by bioadsorption studies with synthetic uranyl solution containing 103 mg U/L in 0.1 M Na₂SO₄. The relatively high sulphate content in these studies was to minimize sulphate concentration effects, since all pH adjustments were done by adding sulphuric acid to the test solution. Using Streptomyces levoris as the test organism the equilibrium bioadsorption capacities on a dry weight basis at 20°C were 47.71 mg U/g at pH=5.0, 28.58 mg U/g at pH=3.0, and 25.59 mg U/g at pH=2.0, respectively. In these experiments, the pH being the only significant variable, the decline of bioadsorption with decreasing pH was unequivocally demonstrated.

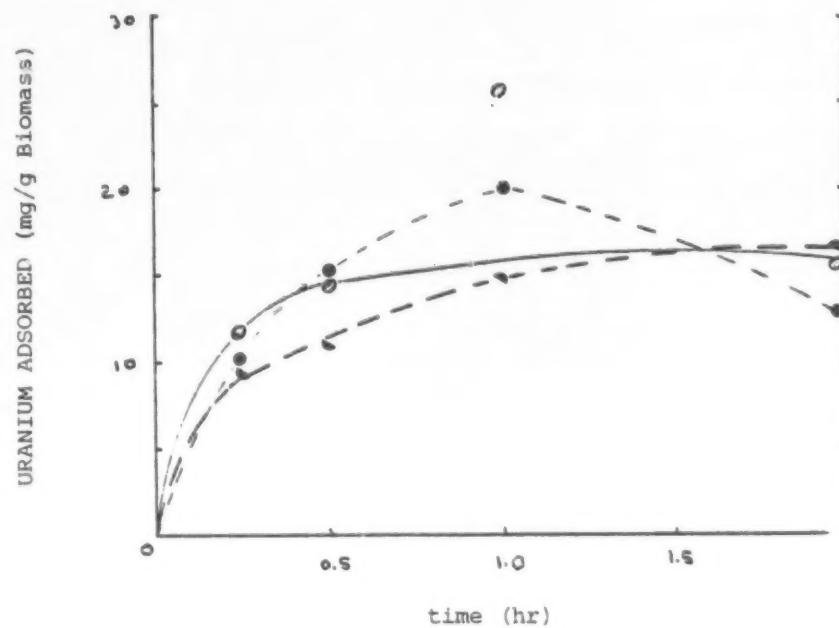
The relatively poor adsorption capacity from process liquors at low pH values (pH=2.0) may have been the result of two factors. First, anionic uranium adsorption sites such as carboxylic groups are protonated at low pH values. In this case, adsorption is dependent on proton exchange with the liquid phase as indicated by the following equilibrium:



As shown above, decreasing pH values (i.e. increasing proton concentrations) do not favour uranyl adsorption from the solution. In addition, low pH values may cause degradation of the adsorbing sites. The decline of uranium bioadsorption with time at low pH values (see Figures 6.2.8 and 6.2.9, for example) offers some evidence for degradation. Electron photomicrographs by Tsezos (1982a) suggest structural damage to cell wall

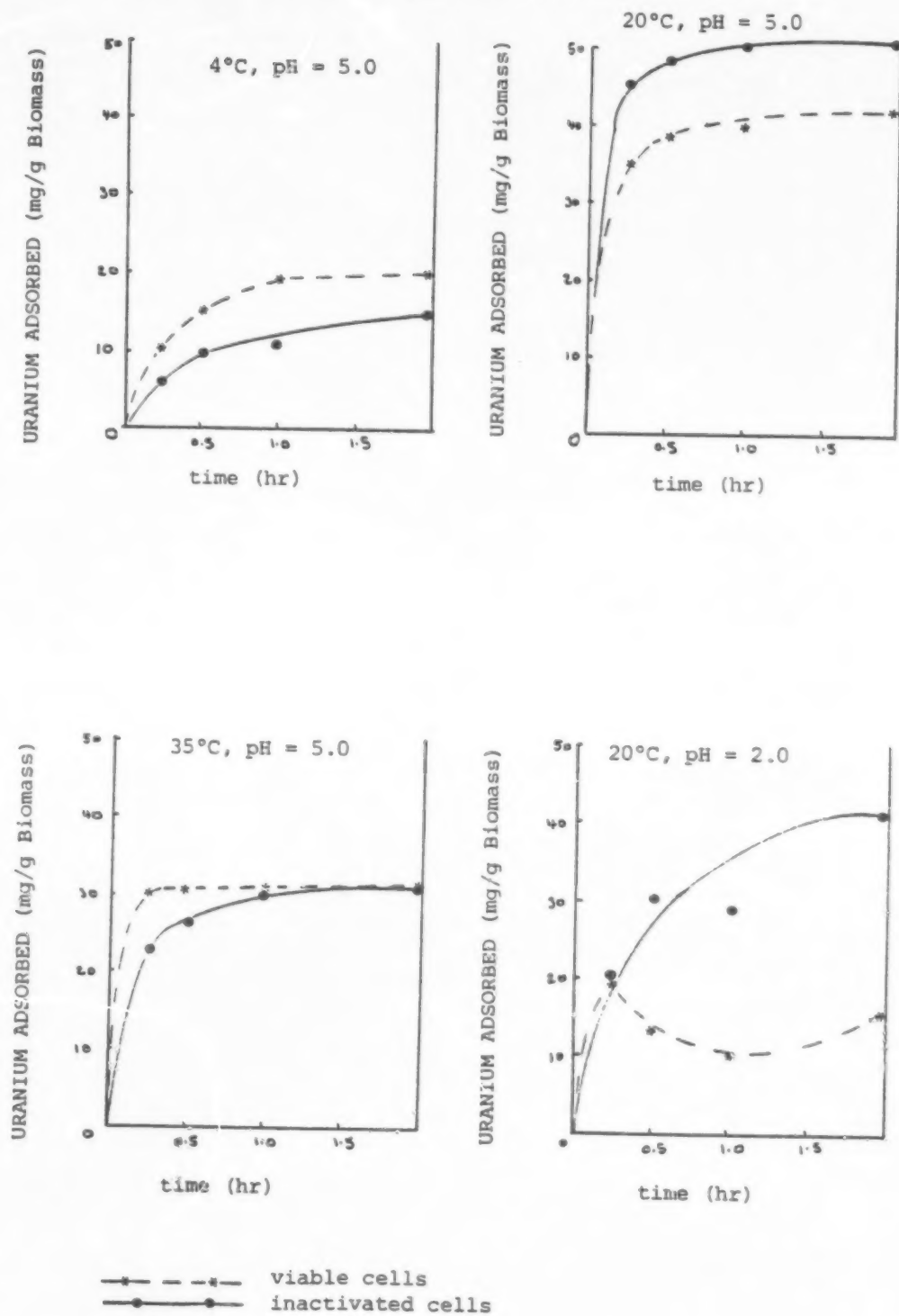


FIGURE 6.2.8 - pH EFFECTS ON INACTIVATED
Rhizopus arrhizus



---●--- pH = 2.0
—○— pH = 3.5
---●--- pH = 5.0

FIGURE 6.2.9 - *Streptomyces levoris* STUDIES
Temperature, pH, Viability Effects



components under low pH conditions and would explain significant desorption by a pH shift only.

Since all bioadsorption studies from process liquor were performed in duplicate, the reproducibility of the bioadsorption capacity could be tested. The standard error in bioadsorption capacity between data pairs after 2 hours exposure (all microorganisms, both viable and inactivated) was found to be 3.38 mg U/g at pH=5.0; 4.44 mg U/g at pH = 3.5, and 10.52 mg U/g biomass at pH = 2.0, respectively. The high standard error, coupled with low bioadsorption at pH = 2.0 reflect the poor reproducibility of the bioadsorption capacity in spite of good experimental techniques.

The effect of the temperature of adsorption on the uranium bioadsorption capacity is shown in Table 6.2.3. In Table 6.2.4, the temperature effect is expressed as the ratio of bioadsorption capacities for viable and thermally inactivated cells.

The comparison of 35°C and 4°C adsorption temperatures is most valid, since the initial uranium concentrations of the pH adjusted process liquor were approximately the same. Comparison and adsorption at 20°C is invalidated by the different initial uranyl ion concentrations. To eliminate the time effects on adsorption, the ratios in Table 6.2.4 are expressed as "equilibrium" bioadsorption capacities. These equilibrium concentrations were estimated from time-dependent adsorption data (see Figures 6.2.9 and 6.2.10) using equation 2.2.18.

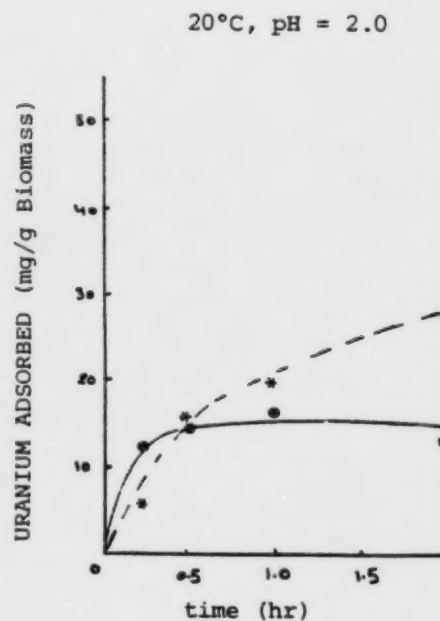
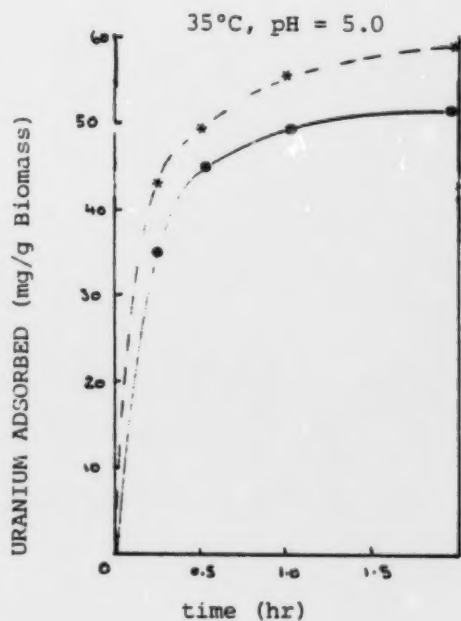
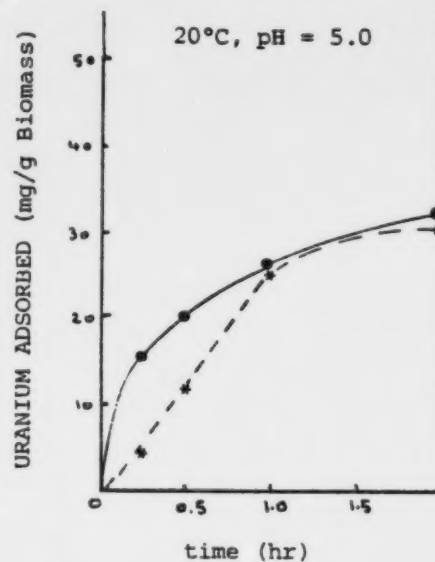
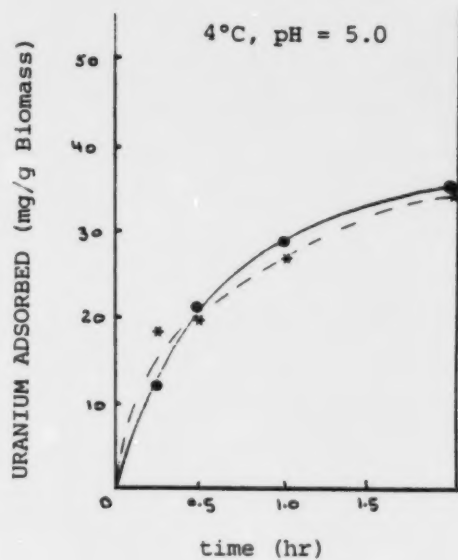
As shown in Table 6.2.4, with the adsorption ratio being generally greater than unity, the bioadsorption capacity increases with increasing temperature. This increase is normally observable in both viable and inactivated suspensions. The

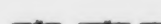
Table 6.2.4

EFFECT OF TEMPERATURE ON URANIUM BIOADSORPTION

<u>SPECIES</u>	<u>URANIUM BIOADSORPTION AT 35°C (mg/g dry weight)</u>	
	<u>URANIUM BIOADSORPTION AT 4°C (mg/g dry weight)</u>	<u>INACTIVATED CULTURE</u>
<u>Rhizopus arrhizus</u>	2.09	1.71
<u>Streptomyces levoris</u>	1.56	2.14
<u>Mixed Culture</u>	1.78	1.40
<u>Chlorella vulgaris</u>	1.98	0.94
<u>Saccharomyces cerevisiae</u>	1.31	1.50

FIGURE 6.2.10 MIXED CULTURE STUDIES
Temperature, pH, Viability Effects



 viable cells
 inactivated cells

increase in adsorption capacity with increasing temperature has been reported previously (Strandberg et al., 1981; Tsezos and Volesky, 1981). The mechanistic significance of this phenomenon is not clear. It would imply, that bioadsorption is an endothermic process, since increasing temperatures favour the equilibrium toward the adsorbed state.

During the course of this study it has become increasingly evident that previously published uranium bioadsorption capacities in synthetic solutions are not achievable in process solutions. Virtually all previous work concerns uranium bioadsorption from uranyl nitrate ($\text{UO}_2(\text{NO}_3)_2$) solutions. Unlike the nitrate which complexes poorly with uranium, the sulphate and uranium in the process liquor form a variety of inorganic complexes. The bioadsorption of these complexes, if they adsorb at all, is likely to differ from the uranyl ion. In addition, the process liquor contains several metal ions at significant concentrations, particularly ferric ion at low pH conditions, which can compete with the uranyl ion for adsorption sites. To shed more light on the adsorption process, a series of experiments were performed with synthetic solutions as described below.

Bioadsorption studies with inactivated Streptomyces levoris as the adsorbing biomass were performed at pH=4 in synthetic UO_2NO_3 solutions (220 mg U/L) containing 0.00 to 0.1 M sulphate added as Na_2SO_4 .

Since the experiments comprised identical initial conditions with respect to both uranium and biomass concentrations and because the only cation, sodium, does not compete with UO_2^{2+} for adsorption sites (Tobin et al., 1984), the parameters of Equation 2.2.16 could be calculated assuming that the maximum adsorption capacity (X_{max}) does not depend on the sulphate level. The parameter estimates the predicted bioadsorption capacities and

the bioadsorption capacities calculated from experimental data are summarized in Table 6.2.5. The good agreement between predicted and "measured" uranium bioadsorption capacities is obvious, particularly after exposure periods of 1 hour or more. The most significant feature is the effect of the sulphate concentration on the half saturation constant at sulphate levels between 0.0 M and 0.06 M sulphate. The increase is attributable to the formation of uranyl-sulphate complexes ($\text{UO}_2\text{SO}_4(\text{aq})$, $\text{UO}_2(\text{SO}_4)_2^{2-}$), which are apparently not adsorbed from the solution. The formation of these sulphate complexes gives rise to an apparent decrease in bioadsorption capacity. It is also evident, that synthetic solutions containing no competing cations for adsorption sites give substantially higher adsorbance than the process liquor. It should be noted, that measured uranium bioadsorption capacities in Table 6.2.5 are at least twice as high as bioadsorption capacities in the process liquor.

The effect of competing cations (metal ions) on uranium bioadsorption by Streptomyces levoris is shown in Table 6.2.6. These adsorption tests were performed in synthetic uranium solutions ($\text{UO}_3(\text{NO}_3)_2$) with 0.1 M Na_2SO_4 . The experiments were up to 30 hours duration. The metal species were added as chlorides. The concentrations of metal ions other than iron correspond to near maximum levels in the process liquor (see Table 6.1.1). Although all metals caused a decline in the adsorption capacity, the adverse effect of the ferric ion on the bioadsorption of uranium by S. levoris is the most remarkable. The adsorption of metal ions other than uranium by Rhizopus arrhizus has been demonstrated by Tobin et al. (1984), while Tsezos and Volesky (1982) have reported the interference of Cu^{2+} , Zn^{2+} , and Fe^{2+} with the UO_2^{2+} adsorption of the same species.

Of all metals tested, Galun et al. (1984) have noted, that the ferric ion (Fe^{3+}) is the most significant with regard to uranium

Table 6.2.5

EFFECT OF SULPHATE CONCENTRATION ON URANIUM ADSORPTION BY Streptomyces levoris

SULPHATE CONCENTRATION MOLES/LITRE

Time (hr)	0			0.02			0.04			0.06			0.08			0.10		
	C_p	X_p	X_M	C_p	X_p	X_M	C_p	X_p	X_M	C_p	X_p	X_M	C_p	X_p	X_M	C_p	X_p	X_M
0.5	29.4	120	110	42.8	112	98	68	96.6	93.9	64	98.7	91.4	67.9	96.3	91.4	72.4	93.4	81.6
1.0	9.84	133	139.7	18.8	127.3	130	35.9	116	122.4	33.3	118.2	123.9	35.6	116.7	124.5	38.8	114	125.3
2.0	2.41	137.7	137.1	8.47	133.9	134.1	18.07	127.8	128.4	17	128.5	125.7	17.5	128.2	127.3	19.1	127	126.5
96.0	1.3	138.4	138.4	6.58	135.1	134.9	12.2	131.5	134.1	12.1	131.5	131.8	11.3	132.1	133.4	11.8	131.8	132

Parameter estimates

$X_{\max}$ (mg/g)	181	181	181
K (mg/L)	0.4	2.2	3.5
k_1 hr ⁻¹ (M/L) ⁻¹	2.8×10^{-2}	2.1×10^{-2}	1.4×10^{-2}
		1.3×10^{-2}	4.2
			1.3×10^{-2}
			4.4
			1.2×10^{-2}

 C_p = predicted concentration of uranium remaining in solution after time t in mg/L X_p = estimated U bioadsorption capacity (based upon C_p) in mg/g biomass X_M = measured bioadsorption capacity by experiment in mg/g biomass C_0 = 220 mg U/L σ = 1.58 g/L

Table 6.2.6

EFFECT OF METAL IONS ON URANIUM
BIOADSORPTION BY Streptomyces levoris

<u>Metal species</u>	<u>Initial Metal Concentration (mg/L)</u>	<u>Uranium Adsorption Capacity (mg/g)</u>	<u>Change in Uranium Bioadsorption (%)</u>
None	-	47.71 (6.11)*	-
Cobalt (Co^{2+})	15	33.08 (1.93)	-30.7
Copper (Cu^{2+})	10	35.65 (3.98)	-25.3
Iron (Fe^{3+})	15	17.21 (3.64)	-63.9
Nickel (Ni^{2+})	15	36.35 (1.64)	-23.8
Zinc (Zn^{2+})	15	35.11 (4.17)	-26.4

pH = 5.0

Initial uranium concentration: 103 mg/L

* Standard error (3 determinations)

bioadsorption by Penicillium digitatum. The effect of ferric ion (as FeCl_3) addition on uranium bioadsorption by Rhizopus arrhizus, Streptomyces levoris, and mixed culture (sewage sludge) is shown in Table 6.2.7. The experiments were performed at suboptimal pH values for UO_2^{2+} adsorption to prevent the precipitation of Fe^{3+} as a hydroxide. Considering changes in UO_2^{2+} and Fe^{3+} concentrations, R. arrhizus and S. levoris may, in fact, preferentially adsorb the ferric ion. The effect of ferric ion on UO_2^{2+} uptake is most pronounced for Streptomyces levoris. It is interesting to note, that the mixed culture (sewage sludge) was loaded with iron prior to testing and then released into the liquid phase or displaced by the UO_2^{2+} at the test pH value. In this case, the apparent adsorption of the ferric ion from the solution was not significant.

6.3 Equilibrium Studies

The experimental results pertaining to this section are tabulated in Appendix B.3. In this section, the effects of the physiological and physical parameters on the maximum equilibrium loading capacity are presented. The physiological parameters include biomass type, viability (vs. thermal inactivation), and physiological age. The physical parameters include the effect of pH.

The equilibrium curves of uranium adsorbed vs uranium concentration are illustrated for Rhizopus arrhizus, Streptomyces levoris, mixed culture and Chlorella vulgaris in Figure 6.3.1 to 6.3.4. From this equilibrium data, maximum adsorption capacities were calculated using both the Langmuir and Freundlich isotherm.

The maximum adsorption capacities are summarized in Table 6.3.1. For the most part, both isotherms fit the data reasonably well. Tzeos and Volesky (1981) report similar goodness of fit. In

Table 6.2.7

EFFECT OF FERRIC ION ON URANIUM BIOADSORPTION - INACTIVATED

BIOMASS	pH	INITIAL (U) (mg/L)	FINAL (U) (mg/L)	INITIAL (Fe) (mg/L)	FINAL (Fe) (mg/L)	U(ADSORBED) (mg/g)
<u>Rhizopus</u>						
	2.7	175	132	0	1.0	16
	2.7	190	131	19	5	20
	2.8	175	123	44	4	20
	2.8	182	139	92	9	16
<u>Streptomyces</u>						
	2.8	175	112	0	0.2	36
	2.8	162	127	16	4	22
	2.8	171	145	43	10	15
	2.8	167	158	83	34	5.2
<u>Mixed Culture</u>						
	2.8	175	47	0	87	91
	2.8	162	96	16	93	51
	2.8	179	125	45	90	37
	2.8	175	123	88	88	37

FIGURE 6.3.1 - EQUILIBRIUM STUDY WITH INACTIVATED
Rhizopus arrhizus, pH 5.0, 20°C

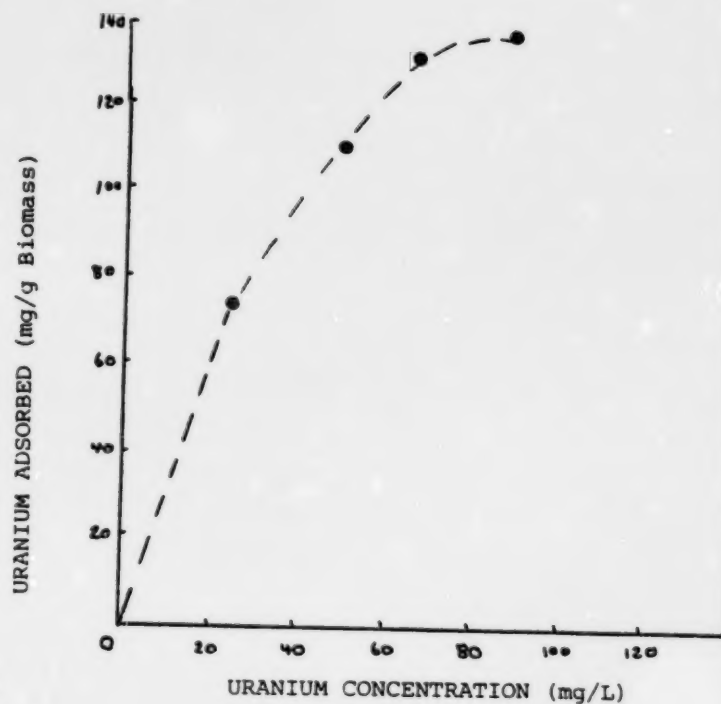


FIGURE 6.3.2 - EQUILIBRIUM STUDY WITH AGED
Streptomyces levoris, pH 5.0, 20°C

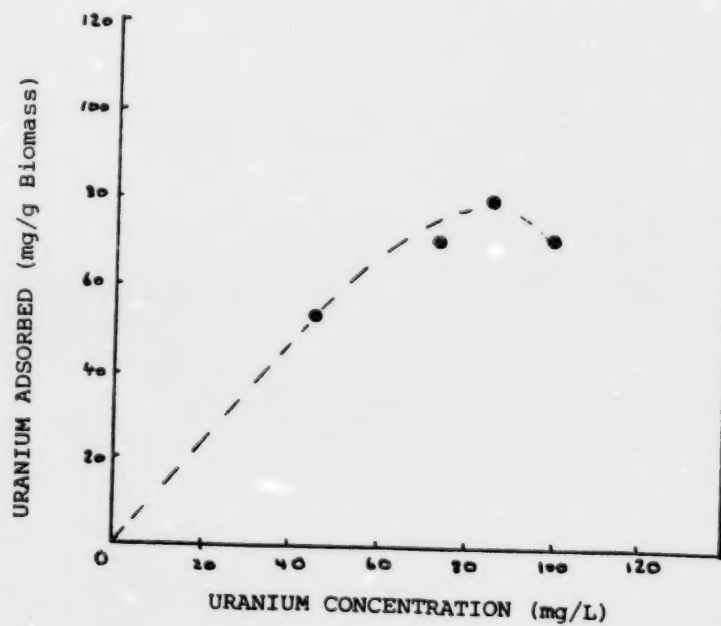


FIGURE 6.3.3 - EQUILIBRIUM STUDY WITH INACTIVATED
MIXED CULTURE, pH 5.0, 20°C

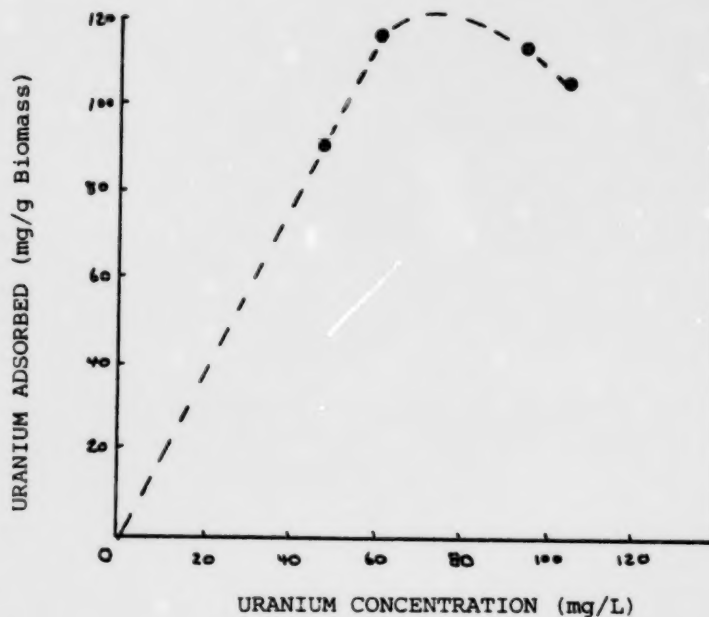
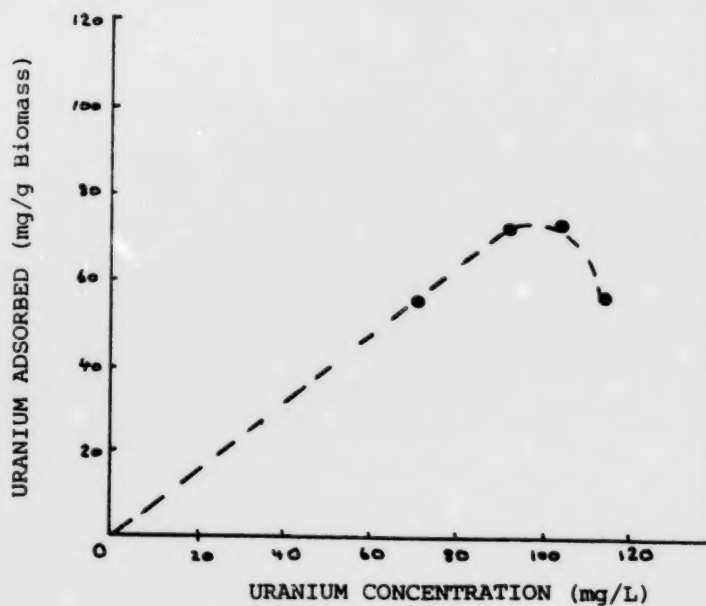


FIGURE 6.3.4 - EQUILIBRIUM STUDY WITH INACTIVATED
Chlorella vulgaris, pH 5.0, 20°C



some experiments (low pH studies), the maximum adsorption capacity was reached and therefore the data is reported as the same for both isotherms. For the Freundlich isotherm data, maximum capacities are based upon a constant level of 243 mg/L uranium to permit comparison of the data.

It is worthwhile to discuss the implications of the maximum adsorption capacities listed in Table 6.3.1 with regard to adsorption from process solutions. It should be noted, that these maximum capacities, calculated from isotherms, are theoretical rather than observable values. Although these maximum adsorption capacities are very comparable with data derived from synthetic solutions, they are attainable only at very high uranium concentrations and are not very useful predictors, by themselves, of the uranium adsorption capacity from actual process solutions. A more useful indicator is a maximum loading capacity, which is dependent on the actual concentration of uranium in the process solution. In addition, an equilibrium uranium capacity may be defined for closed (batch) systems, which is dependent on both the initial uranium concentration and the initial biomass concentration.

The maximum loading capacities and equilibrium adsorption capacities for Rhizopus arrhizus and Streptomyces levoris at high (150 mg U/L) and low (30 mg U/L) uranyl concentrations is illustrated in Table 6.3.2. This table was prepared making use of the Langmuir isotherms derived from adsorption studies in pH adjusted (pH=5.0) process liquors. It is notable, that Streptomyces levoris appears to possess somewhat better adsorption capacities at low uranium concentrations in spite of the higher maximum adsorption capacity of R. arrhizus as shown in Table 6.3.1. At higher uranium levels, a somewhat higher adsorption by Rhizopus arrhizus is predicted. The equilibrium uranium bioadsorption capacities from batch solutions is very

Table 6.3.1

MAXIMUM ADSORPTION CAPACITY (20°C)

SPECIES	pH	MAXIMUM ADSORPTION CAPACITY	
		CALCULATED FROM LANGMUIR ISOTHERM	CALCULATED FROM FREUNDLICH ISOTHERM*
<u>Rhizopus arrhizus</u> (inactivated)	3.5	42.3	42.3
	4.0	49.7	49.7
	5.0	257.7	265.0
<u>Streptomyces levoris</u> (inactivated) inactivated viable inactivated	3.5	62.3	62.3
	4.0	-	159.8
	5.0	120.3	114.3
	5.0	197.2	237.4
Mixed Culture	3.5	46.6	49.9
	4.0	52.5	52.5
	5.0	108.5	108.5
<u>Chlorella vulgaris</u> (inactivated)	3.5	28.5	28.5
	4.0	26.2	26.2
	5.0	63.9	63.9
<u>Saccharomyces cerevisiae</u>	3.5	72.5	50.8

* maximum equilibrium uranium concentration = 243 mg/L U

Table 6.3.2

EFFECT OF BIOMASS CONCENTRATION (σ) AND URANIUM CONCENTRATION
ON THE ADSORPTION CAPACITY

Uranium Concentration (mg/L)	<u>Rhizopus arrhizus</u>			<u>Streptomyces levoris</u>		
	Max. Loading Capacity $X_{\max}$	Equilibrium Capacity σ = 0.5	Equilibrium Capacity (mg/g) σ = 1.0	Max. Loading Capacity $X_{\max}$	Equilibrium Capacity σ = 0.5	Equilibrium Capacity mg/g σ = 1.0
30	81.5	37.7	24.5	87.6	40.57	24.6
150	179.9	141.6	105.2	157.7	135.5	106.2
						14.7

strongly influenced by the concentration of the biomass in the system. It is obvious, that the lower the biomass concentration, the higher is the apparent equilibrium adsorption. Since biomass concentrations used in uranium bioadsorption studies are seldom mentioned any comparison with previously published data is an essentially impossible task. For important conclusion in this regard is the development of standardized procedures for the quantitative and more meaningful evaluation of metal adsorption by microorganisms.

6.4 Batch Uranium Desorption Studies

Test results for the uranium desorption equilibrium are provided in Appendix B-4. The desorbing solutions employed were sulphuric acid, ammonium sulphate, EDTA and sodium bicarbonate.

Previous studies by Galun et al. (1983) had indicated that alkali carbonates and EDTA were effective in stripping uranium from mycelium biomass while aqueous ammonia and organic acids were not effective. Tsezos (1984) evaluated several inorganic acids, ammonium sulphate and carbonate solutions and found similar results.

To assess whether any of the principal biomasses could be stripped with sulphate ion, both weak sulphuric acid at 0.02 N and 0.1 M $(\text{NH}_4)_2\text{SO}_4$ were evaluated. Both these solutions were considered compatible with the potential future underground application at a uranium mine.

The results of the desorption studies are summarized in Table 6.4.1.

As expected, the 0.1 molar sodium bicarbonate was the most effective desorbing agent with removal efficiencies of

Table 6.4.1

URANIUM DESORPTION EQUILIBRIUM			
<u>SPECIES</u>	<u>Rhizopus arrhizus</u>	<u>Mixed Culture</u>	<u>Streptomyces levoris</u>
Uranium adsorbed at pH = 3.5 (mg/g dry wt)	61.0	110.75	76.75
<u>Desorbing Reagent</u>	% Desorption		
H ₂ SO ₄ (0.02N)	38.71%	69.23%	53.85%
pH = 2.05			
(NH ₄) ₂ SO ₄ (0.1 M)	1.15%	0.1%	15.49%
pH = 5.3			
EDTA (0.02 M)	4.25%	10.53%	43.21%
pH = 4.7			
NaHCO ₃ (0.1 M)	78.69%	89.38%	94.81%
pH = 8.2			

approximately 80 percent for Rhizopus arrhizus, 90 percent for the mixed culture and 95 percent for Streptomyces levoris.

EDTA was moderately effective on the Streptomyces recovering 43% of the uranium but proved to be relatively ineffective on the other biomasses (<11% recovery).

Ammonium sulphate at 0.1 M was ineffective at desorbing Rhizopus or the mixed culture but did marginally strip the Streptomyces (15%). Higher concentrations of $(\text{NH}_4)_2\text{SO}_4$ may prove more effective.

The 0.02 N sulphuric acid provided moderate results stripping 38.7 percent from the Rhizopus to 69.2 percent from the mixed culture. Studies with Rhizopus, as reported by Tsezos (1984) have indicated higher H_2SO_4 levels (0.1 to 1 N) can strip more than 80 percent of the uranium from synthetic solutions, however high sulphuric acid levels degrade the cell wall structure.

Based upon these batch stripping studies, it was concluded that NaHCO_3 was the most suitable stripping or desorption agent and as such all future desorption work with columns was confined to using the NaHCO_3 eluent.

Undoubtedly, the good desorption characteristics of NaHCO_3 is due to the very strong complexation of the uranyl ion with carbonate as shown by the equilibrium constants in Table 2.3.2. Sodium bicarbonate is non-destructive with regard to either the structural or the functional properties of the biomass, thus, cyclic adsorption/desorption operations are feasible.

6.5 Column Studies

The experimental results pertaining to the column studies are

provided in Appendix B-5 and a discussion of the results is contained in the following section.

A typical uranium loading curve for Rhizopus arrhizus is shown on Figure 6.5.1. The most remarkable aspect of all uranium adsorption profiles is the apparent lack of sigmoid break-through which is characteristic of ion-exchange columns. This may be attributed to the time-dependent nature of the uranyl ion adsorption on one hand, and the tendency of the biomass to form cellular aggregates on the other hand. The clumping of cells may give rise to the liquid channelling around the biomass flocs. The total uranium adsorbed from the liquid (55.3 mg U/g biomass) was, however, comparable with the maximum adsorption capacity (49.2 mg U/g biomass) found in the equilibrium studies at this pH.

The uranium elution profile with 0.1 M NaHCO_3 reagent is shown on Figure 6.5.2. In many respects, the elution curve is typical of elutions observed with ion exchange resins. In this particular study, 83% of the bioadsorbed uranium was eluted, 69% in the first 60 mL of the eluent.

Table 6.5.1 summarizes the results of cyclic adsorption/desorption column experiments with inactivated Rhizopus arrhizus, Streptomyces levoris and immobilized mixed culture preparations at pH=3.5. In these studies 1 L of uranium process liquor was passed through the column during each adsorption test. The uranium adsorbed by the biomass comprised 69% (Rhizopus), 59% (Streptomyces) and 89% (mixed culture) of the maximum adsorption capacity found by equilibrium studies. The reported adsorption efficiency is the percentage of uranium adsorbed from the test solution. The uranium desorbed readily, particularly in case of Streptomyces and Rhizopus biomasses. Cyclic operation results in a decline of the adsorption capacity for Streptomyces (30%



FIGURE 6.5.1 - LOADING CURVE FOR *Rhizopus arrhizus*
.54 g dry weight, 3 mL/min. @ 188 mg/L-U
pH = 4.0, 20°C

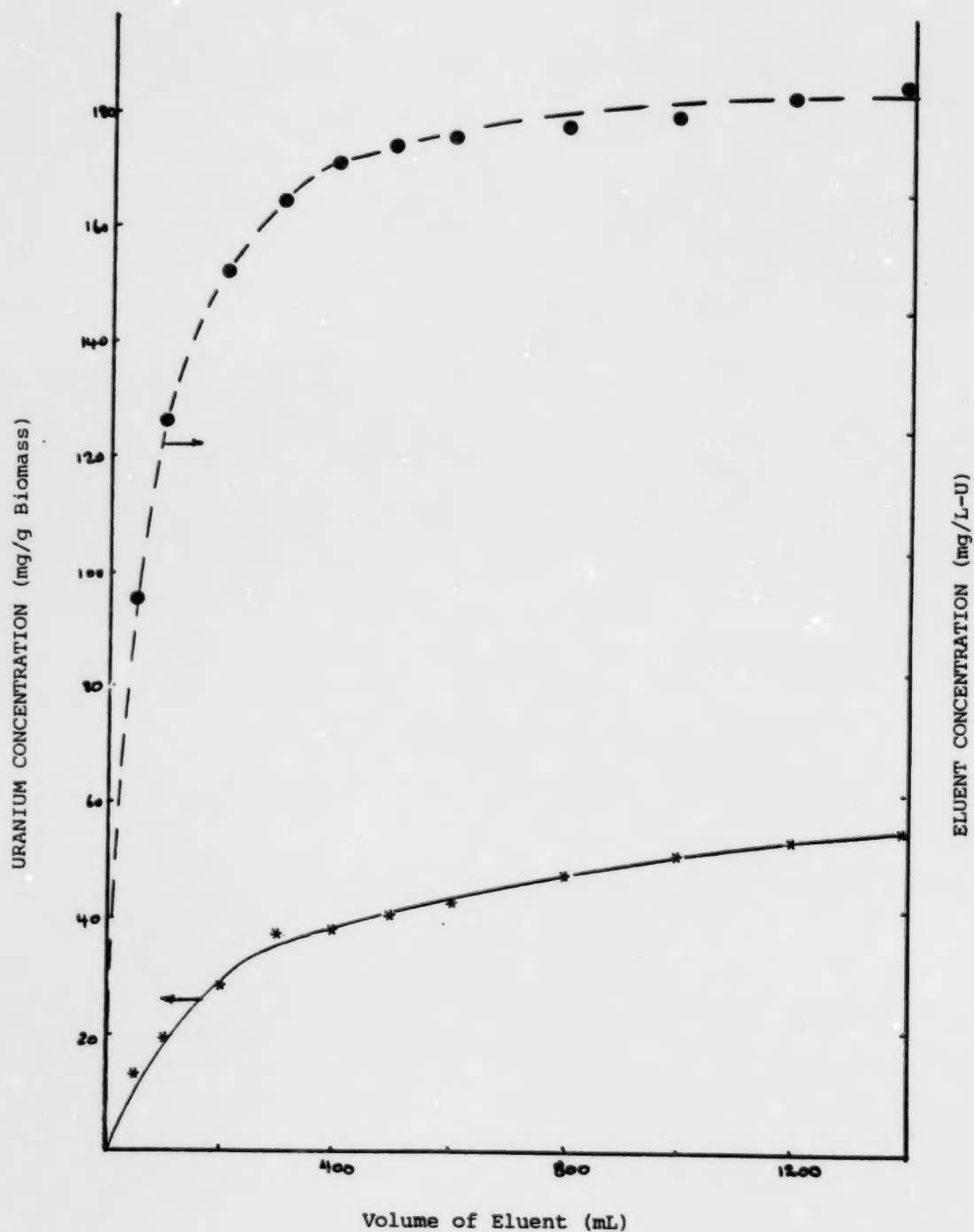


FIGURE 6.5.2 - ELUTION CURVE FOR *Rhizopus arrhizus*
0.54 g dry weight, 0.5 mL/min. 0.1M NaHCO₃,
pH = 8.2, 20°C

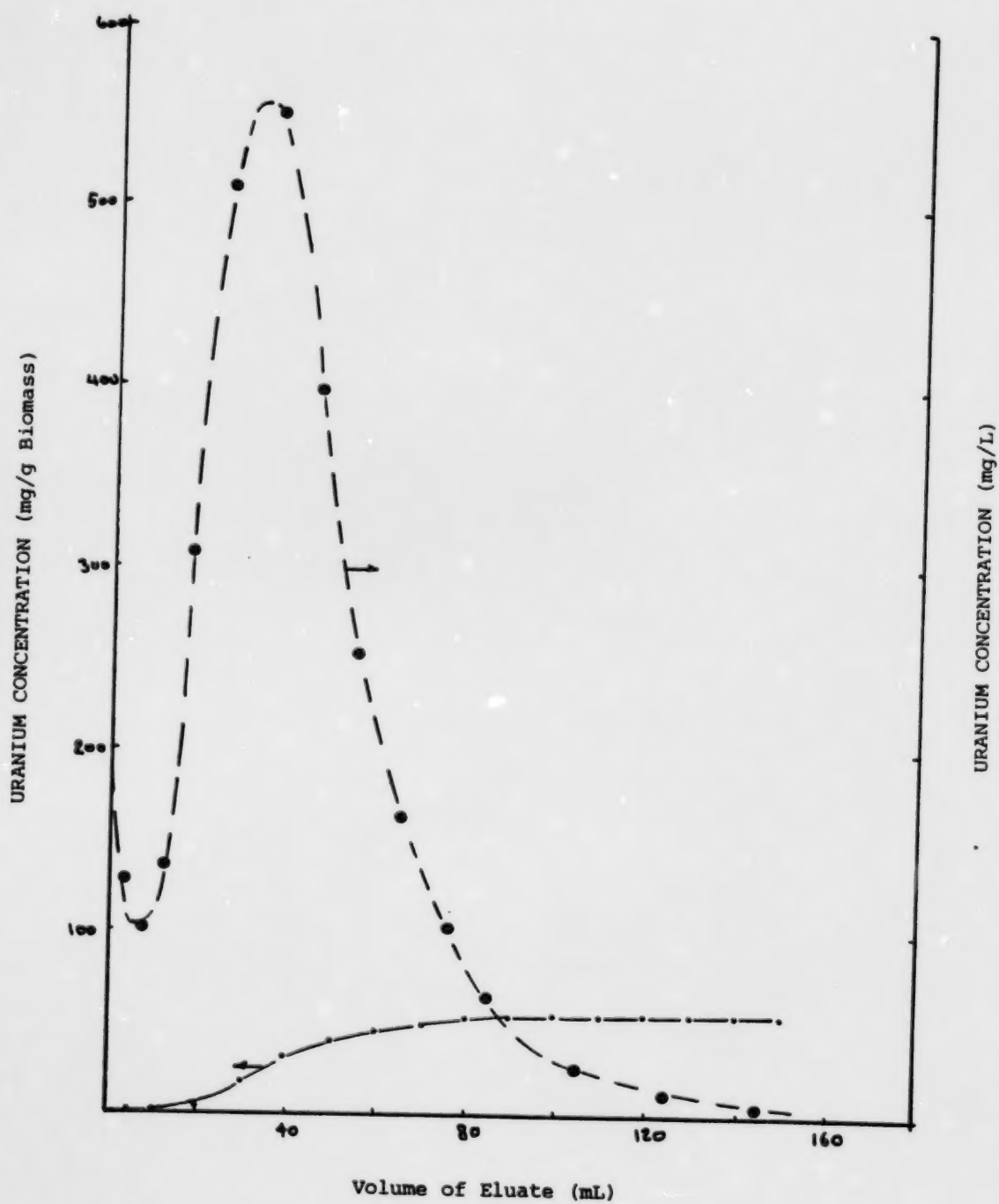


Table 6.5.1

COLUMN ADSORPTION OF URANIUM FROM PROCESS LEACH LIQUOR AT pH = 3.5

	<u>Rhizopus arrhizus</u> <u>(inactivated)</u>	<u>Streptomyces levoris</u> <u>(inactivated)</u>	<u>Mixed Culture</u> <u>(inactivated)</u>
<u>1ST CYCLE</u>			
Uranium Adsorbed (mg/g)	29.04	36.25	41.44
Adsorption efficiency (%)	42.36	32.22	31.23
Uranium desorbed (mg/g)	25.02	33.63	30.84
Desorption efficiency (%)	86.16	92.76	74.42
Concentration factor *	2.28	1.87	1.20
<u>3RD CYCLE</u>			
Uranium Adsorbed (mg/g)	25.02	25.31	45.96
Adsorption efficiency (%)	32.93	20.16	26.65
Uranium desorbed (mg/g)	19.82	24.27	36.33
Desorption efficiency (%)	74.52	95.88	79.22
Concentration factor *	1.93	2.55	1.83

* Concentration factor = $\frac{\text{conc. of U in adsorbate}}{\text{conc. of U in desorbate}}$

decrease); the change in the adsorption capacity of Rhizopus was less pronounced (13.8% decrease); while the adsorption by the mixed culture was virtually unchanged. The small increase noted in Table 6.5.1 is attributable to the uranium retention during the desorption cycle.

The slight decline in the desorption efficiency with Rhizopus arrhizus after the third cycle probably reflects experimental error.

A significant variable on the uranium adsorption capacity was the flow of the process solution through the column. The change of flow rate from 1.5 mL/min to 4 mL/min, for example, resulted in 52% reduction of the column bioadsorption capacity. Since this reduction was believed to be caused mainly by channelling effects, flow studies were conducted by disrupted Rhizopus mycelia. The disruption of the mycelium (Rhizopus) for 7 seconds in the Waring Blender (lower speed) caused substantial compaction in the column and a reduction of the flow from 4.2 mL/min to 0.13 mL/min in a 2 hour period.

During the course of this study, an intensive effort was expended to produce effective, structurally robust immobilized biomass for cyclic column adsorption studies with Rhizopus arrhizus and Streptomyces levoris. These attempts were not successful. Crosslinking with glutaraldehyde reagent produced either crumbling biomass preparations with considerable structural alterations and discoloration (charring) or biomass with poor flow-through characteristics. Biomass that was apparently chemically cross-linked slowly degraded as the testing proceeded. Two columns, however, were operated with immobilized Rhizopus arrhizus and Streptomyces levoris biomasses with partial success at pH=4.0. The adsorption capacities of 28.41 mg U/g (Rhizopus arrhizus) and 26.06 mg U/g (Streptomyces levoris) were



approximately 50% lower than without immobilization. The development of truly robust immobilized cell preparations requires substantially more study with other immobilizing procedures.

7.0 SUMMARY

The principal findings of this study are highlighted in this chapter.

i) BIOMASS GROWTH RATES AND GROWTH YIELDS

- . The fungus Rhizopus arrhizus and the yeast Saccharomyces cerevisiae are very simple to cultivate in axenic culture and produce high growth yields. Rhizopus arrhizus is harvested with ease.
- . Streptomyces levoris is also simple to cultivate, but requires a more complex growth medium.
- . Chlorella vulgaris is a alga that possesses very low specific growth rate and low cell yield. As such it is difficult to produce in large quantities and is subject to contamination. These aspects were troublesome in this study.
- . Air lift fermentation in a continuous fed-batch mode produces high growth yields with minimal problems and would be an excellent method for large scale biomass production.

ii) URANIUM ADSORPTION

- . Rhizopus arrhizus, Streptomyces levoris and the mixed culture proved to be effective biomasses for uranium adsorption. The data in this study suggest that Rhizopus oligosporus may be equivalent or superior to Rhizopus arrhizus.
- . It would appear that there may be many other organisms that

would perform equally as well as the organisms tested in this study.

- . immobilization of cultures coupled with the retention of high bioadsorption capacity is difficult to achieve and many failures can be expected. This area needs further work.
- . bioadsorption capacity is a strong function of pH. Optimum levels which maximize both adsorption capacity and uranium concentration in process solutions would be in the 3.5 to 4 range.
- . thermal inactivation and cell age have only a marginal effect on equilibrium bioadsorption capacity. However, thermal inactivation results in a doubling of the kinetic rate constant for microbes with mycelial growth habits and for sewage flocs. Unicellular microbes such as Chlorella do not appear to be affected.
- . presence of contaminants such as iron, sulphate and heavy metals can greatly reduce bioadsorption capacity. For example, 15 mg/L of ferric ion added to synthetic solutions resulted in 64 percent reduction in Streptomyces levoris bioadsorption capacity. Similarly 15 mg/L of cobalt, copper, nickel and zinc resulted in 31%, 25%, 24% and 26% reductions in bioadsorption capacity respectively.
- . Sulphate levels in process liquor give rise to sulphato complexes which are not adsorbed by any biomass.
- . the adsorption temperature had a marked effect on both viable and thermally inactivated cultures. An increase in temperatures from 4°C to 35°C resulted in 75 percent increase in bioadsorption capacity for viable cultures and a

55 percent increase for inactivated cultures.

- Contact time is an important variable, especially at lower pH values.
- process liquor (mine water) is not suited for direct uranium recovery by biomass. The pH level at 2.0 appears to degrade the cell structure and the associated high contaminant levels (Fe^{3+} for example) result in a marked reduction in bioadsorption capacity.
- bioadsorption data from process liquors is not amenable to direct comparison with published data on synthetic solutions.

iii) URANIUM DESORPTION

- NaHCO_3 is a simple and effective stripping agent for all biomasses tested.
- repeated adsorption/desorption cycles do not greatly effect biomass loading or desorption capacity.
- weak sulphuric acid was marginally effective as a stripping agent while sodium EDTA and ammonium sulphate were ineffective.

iv) MECHANISMS

- simple sorption isotherms such as the Freundlich and Langmuir isotherm describe the experimental data adequately.
- a kinetic model has been proposed to describe the time dependent change of the uranium adsorption capacity. The

model predictions compare favourably with experimental observations.

v) GENERAL COMMENTS

- . data on uranium bioadsorption must be cautiously interpreted. Factors such as temperature, biomass concentration, cell physiology, uranium content in test solutions, contaminant levels, pH, etc. can greatly distort results. Casual observations can lead to erroneous interpretations of the data.
- . Although uranium bioadsorption appear to have a good potential for application with process solutions, this study has not delineated all pertinent factors for optimal design.

8.0 CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

- 1) Many biomasses have excellent capabilities for absorbing uranium. This study has identified Rhizopus arrhizus, Streptomyces levoris and mixed cultures as suitable candidate organisms for future work.
- 2) Other biomasses such as Rhizopus oligosporus may be superior to the organisms tested in this study.
- 3) Temperature, pH and contaminant levels (especially iron) are the primary parameters which effect bioadsorption rates and capacities from process liquor. Viability and cell age are of secondary importance.
- 4) Sodium bicarbonate is an excellent stripping agent for desorption of uranium from biomass.
- 5) Both Langmuir and Fruendlich isotherms adequately describe the equilibrium data. Kinetic equations based upon type adsorption mechanisms suitably model the time dependence of bioadsorption.
- 6) Many cations appear to compete with uranium for adsorption sites. Iron would appear to be the most significant ion in process liquor and appears to be a principal competitor for adsorption sites.
- 7) Process liquor has unsuitable characteristics for direct uranium bioadsorption. Simple treatment via pH adjustment from 2.0 to 3.5 -4 would appear to be adequate to overcome

these conditions.

- 8) Bioadsorption capacities with process solutions are significantly lower than those levels reported for synthetic solutions of uranyl nitrate or chloride.
- 9) Sufficient basic physical and kinetic data are now available through this study to design a small scale plant demonstration facility.

8.2 Recommendations

- 1) Although we are highly encouraged by the results of this study we feel it would be prudent to undertake a comparative study of alternative conventional or non conventional uranium recovery techniques.
- 2) We recommend further basic studies concerning the biopolymers involved in uranium bioadsorption and the positive identification of structural groups responsible for uranium sequestering. These may lead to cell free preparations having enhanced bioadsorption capacity. It could also lead to a more rational screening techniques for microorganisms.
- 3) We recommend that further study into the effects of growth media on bioadsorption capacity and into the testing of alternative cost effective media.
- 4) We recommend that further studies be conducted on the development of robust immobilized biomass that retain high bioadsorption capacity at reasonable flow rates.

- 5) Based upon the results of this study we recommended further work on a small pilot plant scale.
- 6) In conjunction with pilot plant work we recommend the development of standardized procedures for screening biomass for the recovery of any metal. The current variety of techniques being used does not allow any meaningful comparison of reported bioadsorption capacities.

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APPENDIX A

TERMS OF REFERENCE

File No: 26SQ.23440-4-9172
Serial No:

APPENDIX "A"

STATEMENT OF WORK

Requirement:

On behalf of the Department of Energy, Mines and Resources, the contractor will conduct the necessary laboratory investigations and experimentations to determine the best biomass for recovery of uranium from process streams. The report issued under this contract shall be based on a critical evaluation of the results obtained in the laboratory.

Background:

A critical review of the literature performed under contract (DSS No. 26SQ23440-3-9139) identified several possible biomass types for recovery of uranium from solutions. CANMET wishes to confirm, with data generated from laboratory experiments, on actual solution, the ranking of these biomasses identified in the report.

A copy of report no. 26SQ.23440-3-9139 is provided as an attachment.

Objective:

By the generation of laboratory data determine the ranking of the various types of biomass listed below:

(Refer to DSS report No. 26SQ.23440-3-9139)

- (a) Fungal mycelium;
- (b) Microbial derivatives (immobilized cells);
- (c) Mixed microbial (activated sludge) cultures;
- (d) Yeasts or defined microbial cultures; and
- (e) Algae.

WORK STATEMENT

The contractor will perform the work in stages as detailed below:

Stage 1:

The contractor will submit to the Scientific Authority a work plan giving the details of the proposed investigations including the organisms and their source.

General Guidelines for the Work Plan:

The biomasses to be tested should be from the following:

- (a) Fungal mycelium;
- (b) Microbial derivatives (immobilized cells);
- (c) Mixed microbial (activated sludge) cultures;
- (d) Yeasts or defined microbial cultures; and
- (e) Algae.

Each biomass should be investigated for:

- (a) the rate of uranium accumulation;
- (b) the equilibrium distribution of uranium between cell and solvent phases;
- (c) the effect of process variables such as pH, temperature, initial concentration of uranium, the presence or absence

APPENDIX "A" - page 2

- (d) of other cations and anions;
 - (e) the rate of desorption of the accumulated uranium;
 - (f) the equilibrium distribution of the uranium between cell and liquid phases after desorption treatment;
 - (g) any change in the rate of accumulation or the equilibrium distribution ((a) and (b) above) when the biomass is repeated for accumulation of uranium;
 - (h) the effect of pretreatment of the biomass (for example with EDTA) on second and subsequent use (of the biomass) for uptake of uranium;
 - (i) the physical characteristics and their variation with repeated absorption-desorption cycles;
 - (j) ease of generation of biomass supply;
 - (k) any other criteria which the contractor considers relevant;
- and (k) each biomass must be evaluated on actual leach solutions supplied by CANMET.

The contractor shall not proceed to Stage 2 without written confirmation from the contracting authority.

WORK STATEMENT

Stage 2:

On approval of the work plan submitted, the contractor shall carry out the work detailed. Monthly progress reports describing the experiments and the results obtained shall be submitted to the Scientific Authority. The final report in fulfillment of this stage should include all the investigations carried out, conclusions drawn from the results, a tabulated presentation of all material facts and a ranking of the various types of biomass used.

Stage 3:

Perform an evaluation of the five biomasses based on the findings of Stage 2 and any other relevant data available in the literature.

The evaluation should consider such factors as:

- (a) ease of scale-up;
- (b) culture requirements and cost of nutrients;
- (c) suitability of the biomass for use in conventional solid-liquid contacting equipment;
- (d) suitability of each biomass should be evaluated against the most appropriate solid-liquid contacting system;
- (e) most probable economic hurdles;
- (f) most probable major technical problems that are likely to occur; and
- (g) any other factors the contractor identifies as important.

The report should clearly identify each of the criteria listed above.

All experimental work shall be conducted on process liquid supplied by CANMET.

APPENDIX B-1

Typical Examples of Experimental Notes and Data for Growth Experiments

1. Batch reactor data for Streptomyces levoris (Marubishi Fermentor)
2. Batch-fed reactor data for Rhizopus arrhizus (Air Lift Fermentor)
3. Batch fed reactor data for Streptomyces levoris (Air Lift Fermentor)

TABLE-1

Batch Reactor data for the growth of Streptomyces levoris
in a Glucose medium (Temp. = $30 \pm 1^\circ\text{C}$, Reactor Volume = 15 l.
Initial pH = 6.3, Inoculum Vol. = 10%)

SL. No.	Culture time, h.	pH	Glucose Conc.		Dry Cell Wt. x (g/l)	x/x ₀	$\ln \frac{x}{x_0}$
			O.D. * (at 600 nm)	Glucose Conc. (g/l)			
1	0	6.33	0.246	9.84	0.350	1	0
2	3	6.38	0.255	10.2	0.360	1.02	0.0198
3	6	6.41	0.280	11.2	0.363	1.037	0.0363
4	9	6.45	0.313	12.52	0.526	1.502	0.406
5	12	6.73	0.343	13.72	1.256	3.588	1.277
6	15	7.40	0.290	11.6	1.624	4.64	1.534
7	18	7.50	0.283	11.32	2.340	6.68	1.899
8	21	7.70	0.255	10.2	2.389	6.82	1.920
9	24	7.80	0.233	9.32	2.486	7.102	1.960
10	27	7.90	0.226	9.05	3.267	9.334	2.232
11	30	7.95	0.220	8.82	2.925	8.357	2.123
12	33	8.00	0.215	8.62	2.875	8.214	2.105
13	36	8.05	0.211	8.44	2.895	8.271	2.112
14	39	8.05	0.092	3.74	3.112	8.891	2.185
15	42	8.05	0.049	1.962	2.890	8.257	2.111
16	45	8.4	0.024	0.962	2.850	8.14	2.097
17	48	8.4	0.022	0.855	2.935	8.285	2.126

* dilution factor = 8

TABLE-2

EFFECT OF VARYING TRYPTON CONCN. ON THE
GROWTH OF Streptomyces bevoris in a Glucose med.
(shake flask study Temp. = 30°C, Broth vol. = 0.2 l
Inoculum vol. = 5%).

Sample NO.	Culture time (h)	pH	Glucose concn. g/l	Dry cell Wt. (g/l)
<u>Trypton (2 g/l)</u>				
1	16	7.12	9.1	0.365
2	30	7.40	8.35	0.495
3	45	7.29	7.31	0.452
4	45	7.29	7.37	0.462
<u>Trypton (5 g/l)</u>				
1	16	7.22	8.35	0.940
2	30	7.45	6.22	1.135
3	45	7.38	4.48	1.075
4	45	7.37	2.42	1.065
<u>Trypton (10 g/l)</u>				
1	16	7.26	5.21	1.2575
2	30	7.56	5.45	2.755
3	45	7.43	2.32	2.9125
4	45	7.44	2.57	2.9250
<u>Trypton (15 g/l)</u>				
1	16	7.28	8.15	2.015
2	30	7.6	5.22	4.2125
3	45	7.75	0.84	3.975
4	45	7.76	0.64	3.950

RUN #1

ANALYSIS OF EXPT'L DATA ;

Batch Reactor experimental data obtained on Masubishi (15l.) fermenter for 'Streptomyces leuoris' growth in the following medium is given in Table-1.

Medium:

Glucose = 10 gl⁻¹

Tryptone = 15 gl⁻¹

FeSO₄.7H₂O = 0.001 gl⁻¹

Conditions ; Temp. = 30 ± 1 °C

Initial pH = 6.3

Inoculum Vol. = 10%

If 'X' is the cell mass concn. (gl⁻¹) & 'S' is the limiting substrate concn.

Yield of Cell growth $Y_{x/s} = \frac{\Delta x}{\Delta s}$

from Table-1

$$\begin{aligned} \frac{\Delta x}{\Delta s} &= \frac{(3.112 - 0.35)}{(9.84 - 0.88)} \\ &= \frac{2.762}{8.96} = 0.308 \end{aligned}$$

$$Y_{x/s} = 0.308$$

Max^m Sp. Growth rate ; μ_{max} .

Sp. growth rate of the cells, μ is defined as

$$\mu = \frac{1}{x} \frac{dx}{dt}$$

$$\text{or } \frac{dx}{x} = \mu dt$$

$$\ln x \Big|_{x_0}^x = \mu \int_0^t dt$$

$$\text{or } \boxed{\ln \frac{x}{x_0} = \mu t}$$

$$\text{OR } \ln x = \mu t + \ln x_0$$

which implies that if we plot $\ln \frac{x}{x_0}$ Vs t data, it should result in a straight line (in the exponential growth region) and slope of this line will give the max^m μ value.

μ_{max} CALCULATION;

From Fig. 2, a plot of $\ln \frac{x}{x_0}$ Vs t data

We obtain

$$\mu_{\text{max}} = 0.122 \text{ hr}^{-1}$$

SHAKE FLASK STUDY;

RUN #2

In order to see the effect of varying Tryptone (i) Concⁿ on the growth of 'Streptomyces leuorisi' in a medium of (10 gl⁻¹ glucose concⁿ, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O} = 0.001\%$) Contg. 2, 5, 10 and 15 gl⁻¹ of Trypton, Shake flask study was carried out.

Condⁿs:

$$\text{Temp.} = 30 \pm 1^\circ\text{C}$$

Inoculum = 5% (100 cc. medium in 250 cc. flasks)

- (ii) For each set of Trypton Concⁿ (2, 5, 10 & 15 g/l) four samples were taken at 16, 30, 45th hrs (two at 45th hr.) during culture cultivation, and were analyzed for dry cell wt. & glucose concⁿ. pH values were also measured at each time.

Profiles for pH, ^{& Glucose Concⁿ.} dry cell wt. are shown in fig. 3 & 4 which indicate that Trypton Concⁿ of 15 g/l provides much improved growth characteristics & higher cell yield.

BATCH REACTOR DATA FOR THE GROWTH OF *Streptomyces leuostis*
 using Glucose medium
 (Temp. = 30.5°C, V = 15 l.)

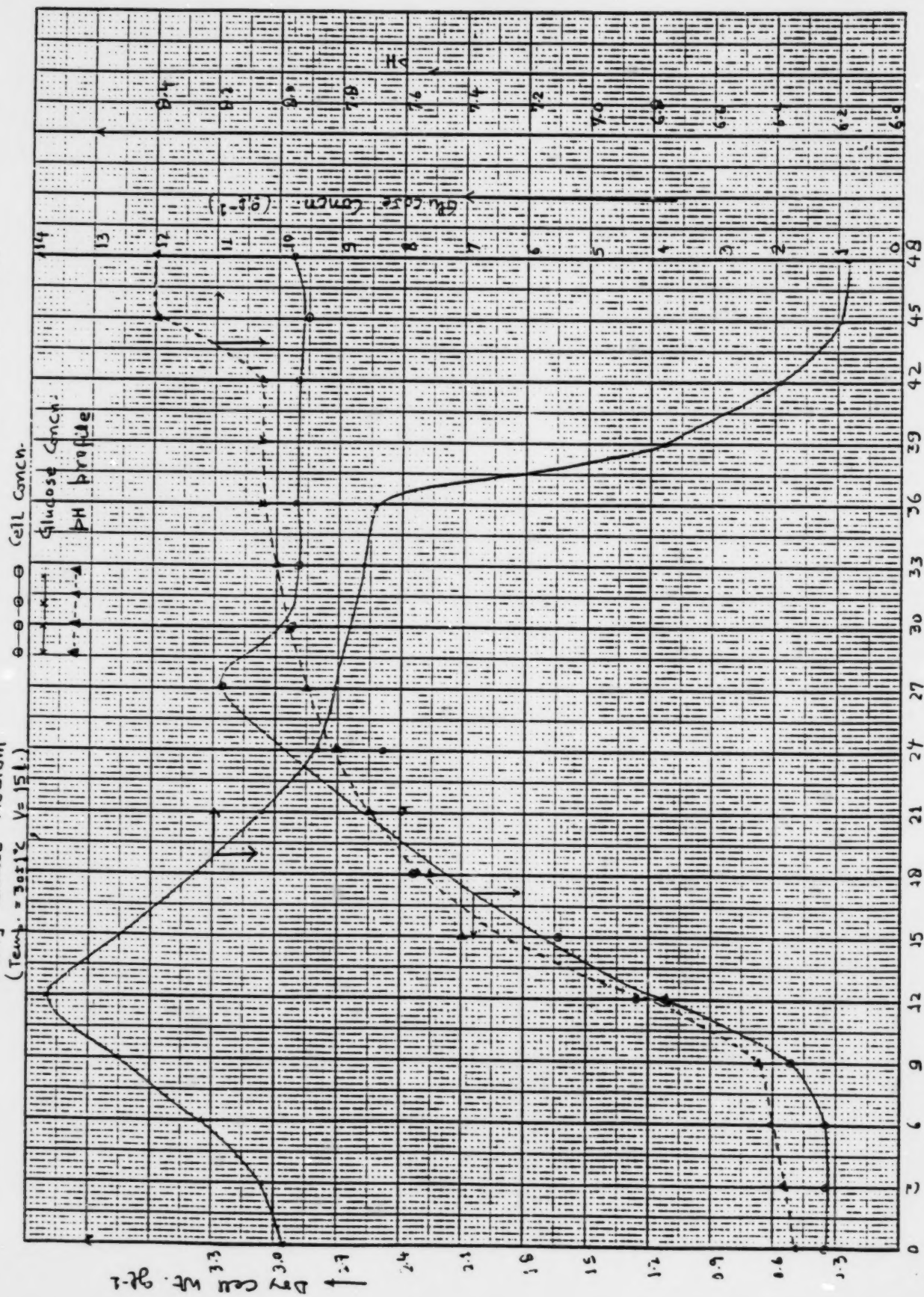


Fig. 1

→ Culture time (hr)

Estimation of μ_{max} . For the Growth of *Schizosaccharomyces lewisii* in a Glucose medium contg. 15g/l Tryptone & 10g/l of Glucose

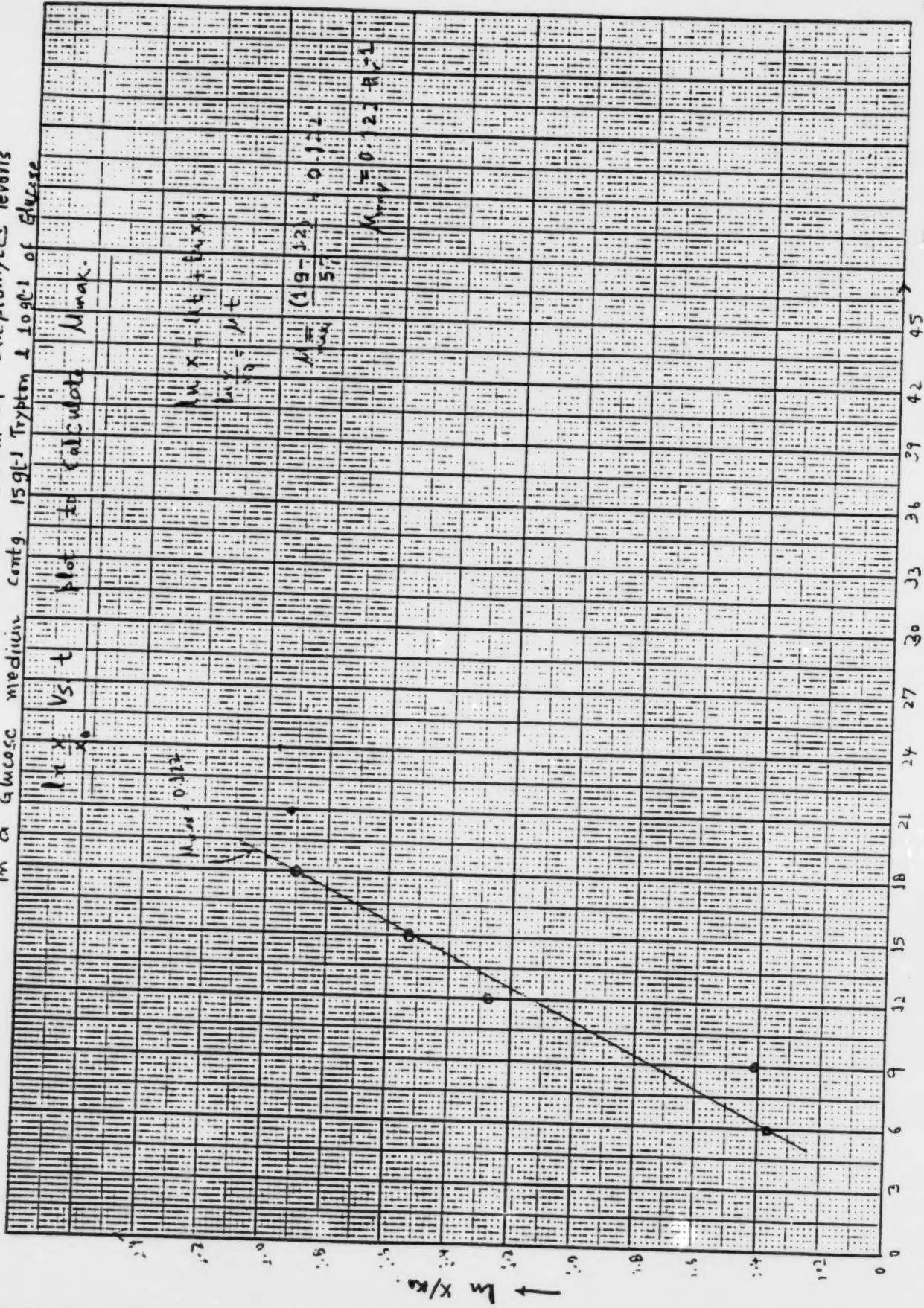


Fig. 2

EFFECT OF VARYING TRYPTON CONCN. ON THE GROWTH OF

Streptomyces *revivis* in a
(Temp = 30.1°C, V = 0.10)

Glucose medium

(cell concn
in profile)

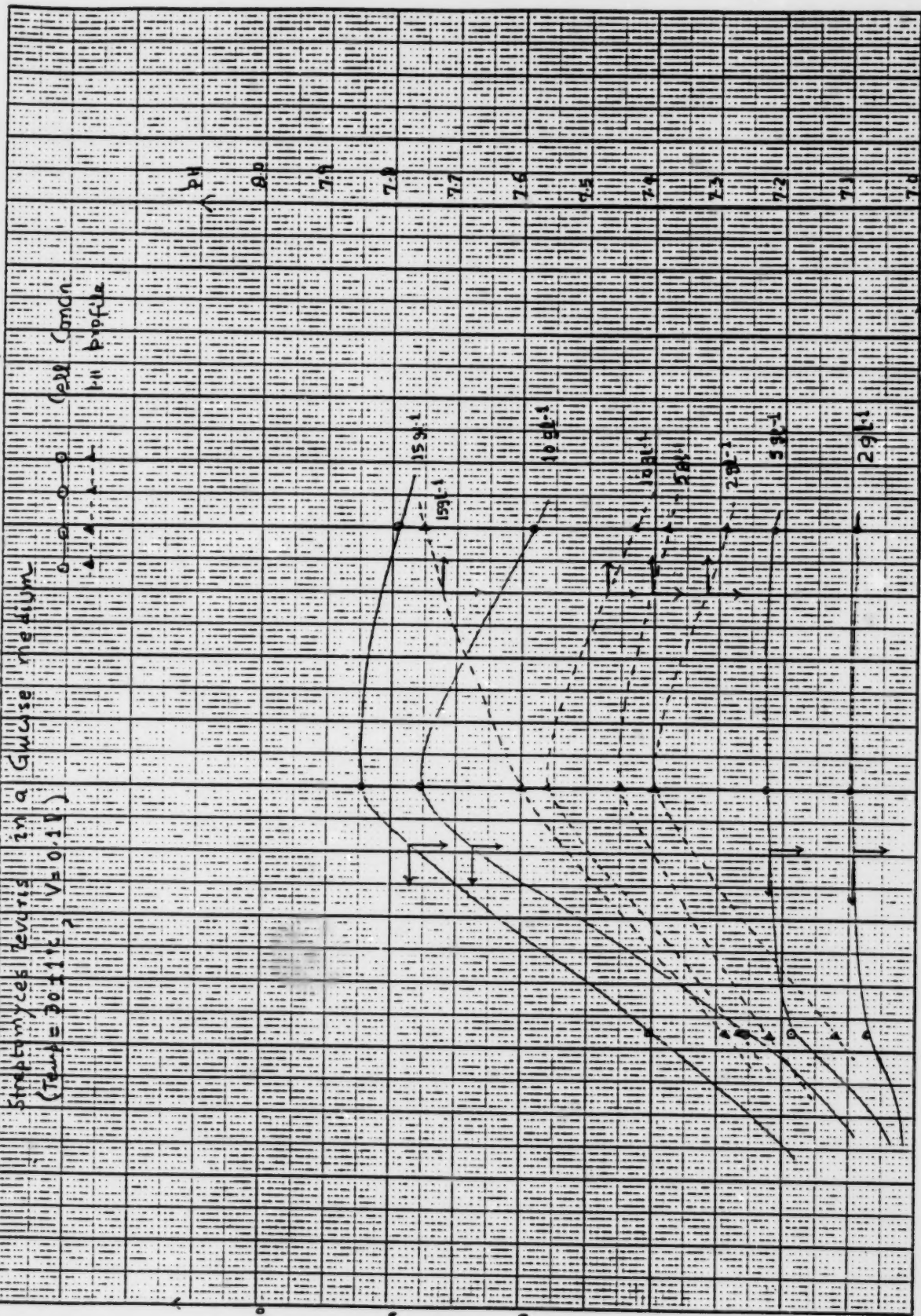
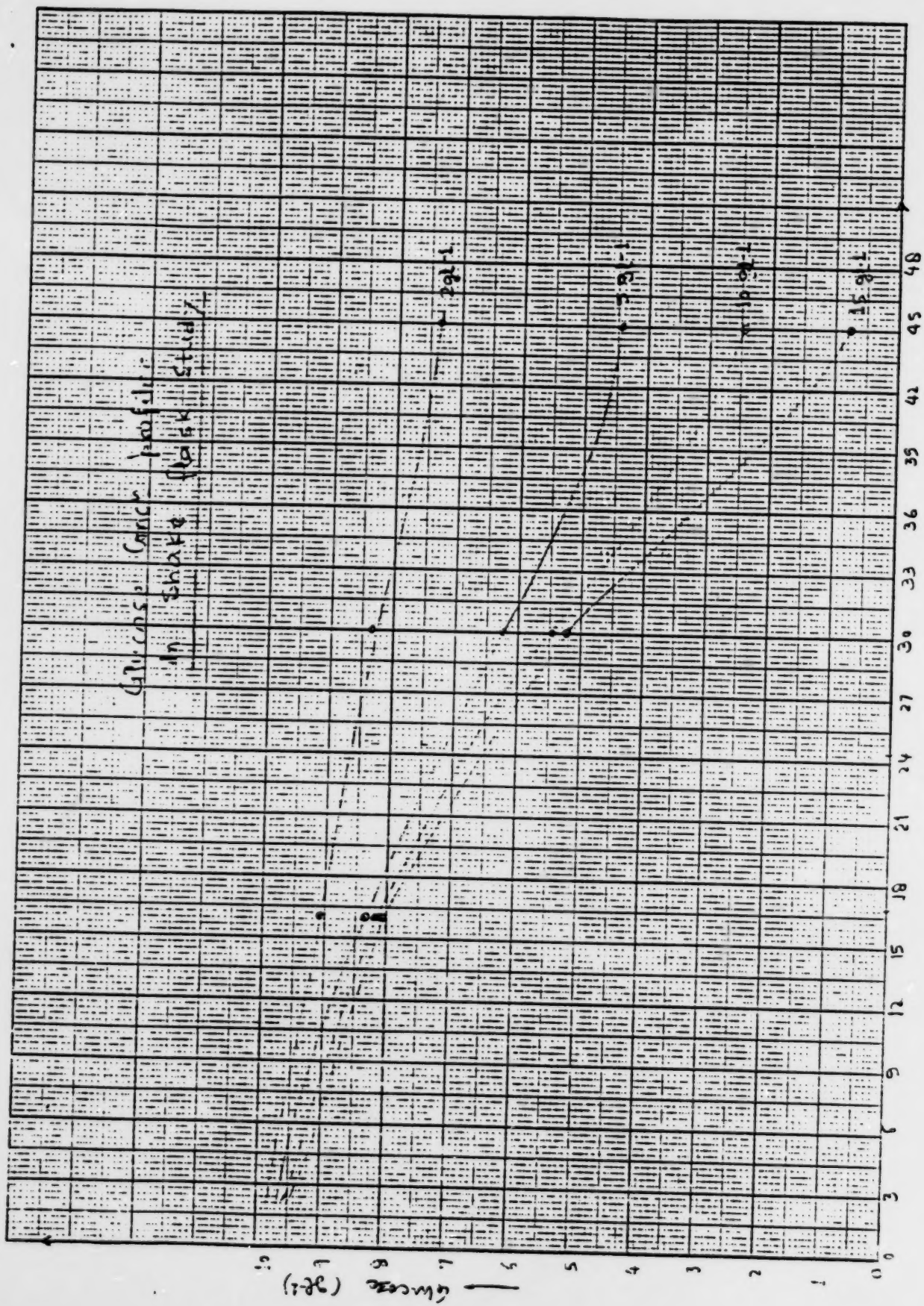


Fig-3

→ Culture time (hrs.)



→ t (hrs)

Fig. 4

FED-BATCH STUDIES ON AIR LIFT FERMENTOR (TEST RUN)

Growth of Rhizopus Arrhizus in a Glucose medium. (Temp. = $30 \pm 1^\circ\text{C}$, Initial reactor vol. = 11 l. Inoculum vol. = 10%)

Sl. No.	Culture time h.	Glucose Conc ⁿ	
		OD * at 600nm	Conc ⁿ gl ⁻¹
1	0	0.095	7.378 **
2	5	0.071	5.514
3	26	0.0075	0.582
4	48	0.0055	0.427
5	24, 72	0.012	0.932

Feed No. 1
(2 litre) →

* dilution factor = 16

** During medium sterilization (partly by direct steam injection) initial substrate Concⁿ fall from 10 gl⁻¹ to 7.378 gl⁻¹.

FED-BATCH EXPERIMENT FOR GROWING RHIZOPUS (AIR LIFT FERMENTOR)

<u>Sample NO</u>	<u>Culture time</u> (hrs)	<u>OD</u> (Gau/ml)	<u>Glucose</u> gl ⁻¹	<u>Vol of liq.</u>
→ 6	72 (29/3, 11.00 AM)	0.0105	0.8115	14 l
7	84	0.051	3.94	
→ 8	96	0.009	0.695	18 l
9	120	0.006	0.463	22 l
10	144	0.007	0.541	26 l

Addition every day

4L feed

Glucose addition

$$4 \times 7.378 = 29.512 \text{ g/d}$$

Reactor vol = 14L

Reactor glucose conc = 0.8115 (before addition)

$$\text{Glucose conc after addition} = \frac{0.8115 \times 14 + 29.512}{18} = 2.27 \text{ gL}^{-1}$$

after 24 h glucose conc = 0.695 gL^{-1}

$$\begin{aligned} \text{Substrate consumption rate} &= \frac{2.27 - 0.695}{24} = 0.0656 \text{ gL}^{-1} \text{ h}^{-1} \\ &\approx 1.575 \text{ gL}^{-1} \text{ d}^{-1} \end{aligned}$$

Reactor glucose conc after addition of fresh 4L feed

$$= \frac{18 \times 0.695 + 29.512}{22} = 1.91 \text{ gL}^{-1}$$

after 24 h, glucose conc = 0.463 gL^{-1}

$$\begin{aligned} \text{Substrate consumption rate} &= \frac{1.91 - 0.463}{24} = 0.0603 \text{ gL}^{-1} \text{ h}^{-1} \\ &= 0.06 \text{ gL}^{-1} \text{ h}^{-1} \end{aligned}$$

U conc

$$\begin{aligned}\text{Total substrate added} &= 10\text{L} + 2\text{L} + 4\text{L} + 4\text{L} \\ &= 20\text{L} \quad (+2\text{L inoculum})\end{aligned}$$

$$\text{total glucose in} = 20 \times 7.378 = 147.56\text{ g}$$

$$\begin{array}{l}\text{glucose left} \\ \text{in the reactor}\end{array} = 22 \times 0.695 = 15.29\text{ g}$$

$$\text{Glucose utilised} = 132.27\text{ g}$$

$$\text{cell conc} = 3.02\text{ gL}^{-1}$$

$$\begin{aligned}\text{cell mass produced} &= \cancel{22 \times 3.02} - \cancel{18 \times 2.98} \quad 22 \times 3.02 \\ &\quad - 2 \times 3 \text{ (inoculum)} \\ &= 60.44\text{ g}\end{aligned}$$

$$\text{Overall cell yield} = \frac{60.44}{132.27} = \underline{\underline{0.457}}$$

cell yield during between two two feeding

$$X_1 = \text{cell conc} = \underline{3.02\text{ gL}^{-1}} \quad \text{in } 22\text{L}$$

$$X_2 = \text{cell conc} = \underline{2.98\text{ gL}^{-1}} \quad \text{in } 18\text{L}$$

$$\text{cell produced} = 22 \times 3.02 - 18 \times 2.98 = 12.68\text{ g}$$

$$\text{Glucose consumption} = 18 \times 2.27 - 22 \times 0.695 = 25.57$$

$$\text{cell yield} = \frac{12.68}{25.57} = \underline{\underline{0.496}}$$

Feed batch bioreactor operation notes

TABLE-1

VALUES OF pH, DRY CELL WT. (X , g/l), GLUCOSE CONC. (g) FOR THE GROWTH OF '*Streptomyces laevis*' in a fed-batch REACTOR OPERATION ($T = 30 \pm 1^\circ\text{C}$, $V = 15\text{L}$, Initial pH = 6.3, Inoculum Vol. 10%).

Sample No.	Culture time, h	pH	Glucose concn g/l (g)	Dry Cell wt. g/l (x)	X/X_0	$\ln \frac{X}{X_0}$
1	0	6.3	0.31	0.100	1	0
2	3	7.1	0.62	0.125	1.25	0.223
3	6	7.4	0.05	0.3875	3.875	1.354
4	9	7.6	0.84	0.63	6.3	1.840
5	12	7.85	8.60	1.1325	11.325	2.427
6	15	8.05	8.42	1.1975	11.975	2.482
7	18	8.25	8.32	1.36	13.6	2.610
8	21	8.28	8.22	1.38	13.8	2.624
9	24	8.30	7.09	1.395	13.95	2.635
10	27	8.25	6.72	1.605	16.05	2.775
11	31:15	8.24	6.12	17.925	17.925	2.886

3 litres total:
 $\Rightarrow$ First batch of substrate added (Glucose added = 21.63 g/l, Trypton = 32.4 g/l - 16.2 g/l)
 0.09216 g

12	31:30	7.75	0.04	1.4925	1	0
13	34	7.9	5.473	1.5875	1.063	0.061
14	40	8.15	6.857	1.900	1.273	0.2414
15	46	8.10	5.35	2.3375	1.966	0.448
16	52	7.96	3.68	3.315	2.221	0.798
17	58	7.88	3.24	3.405	2.281	0.824

TABLE-1 (CONTD.)

⇒ 2nd Batch of substrate added { 3 litres; 21.6 g/l Glucose
32.4 g/l - substrate. $\frac{21.6}{32.4} = 0.667$

Sample No.	Culture time	pH	Glucose concn g/l	Dry cell wt. g/l	$X_1 \times 10^3$	$\ln \frac{X_1}{X_0}$
18	58:15	7.58	6.84	2.7375	1	0
19	61:00	7.74	5.013	3.0350	1.1006	0.0958
20	67:15	7.86	2.87	3.4500	1.262	0.2327
21	73:00	8.08	1.25	3.6675	1.330	0.285
22	79:00	8.25	1.179	4.215	1.525	0.421
23	83:45	8.34	1.03	3.645	1.321	0.279
24	88:00	8.36	1.03	3.635	1.315	0.2762

Fermentor broth volume before 1st feed of
Substrate = 12 litres

Fermentor broth volume before 2nd feed
of Substrate = 12 litres

ESTIMATION OF μ_{max} & $Y_{x/s}$

From fig 3, $\ln x/x_0$ vs t plot

Sp. growth rate of the cells ' μ ' is defined as:

$$\mu = \frac{1}{x} \frac{dx}{dt}$$

$$\frac{dx}{x} = \mu dt$$

$$\ln x \Big|_{x_0}^x = \mu \int_0^t dt$$

or

$$\boxed{\ln \frac{x}{x_0} = \mu t}$$

$$\text{or } \ln x = \mu t + \ln x_0$$

Which implies that $\ln \frac{x}{x_0}$ vs t plot should result in a st. line (in the exponential growth) and slope of this line will give the μ_{max} .

From fig-3 $\Rightarrow$

$$\boxed{\mu_{max} = 0.208 \text{ hr}^{-1}}$$

CALCS for Growth Yield $Y_{x/s}$ $\Rightarrow$

From Table-1 (for growth before adding 1st batch of s)

$$\Delta x = 1.7925 - 0.1 = 1.6925$$

$$\Delta s = 9.94 - 6.12 = 3.82$$

$$\therefore Y_{x/s} = \frac{\Delta x}{\Delta s} = \frac{1.6925}{3.82}$$

$$Y_{x/s} = 0.443$$

prior to 1st feed

For growth after adding 1st lot of feed (substrate)

$$\Delta x = 3.405 - 1.4925 = 1.9125$$

$$\Delta S = 9.04 - 3.24 = 5.8$$

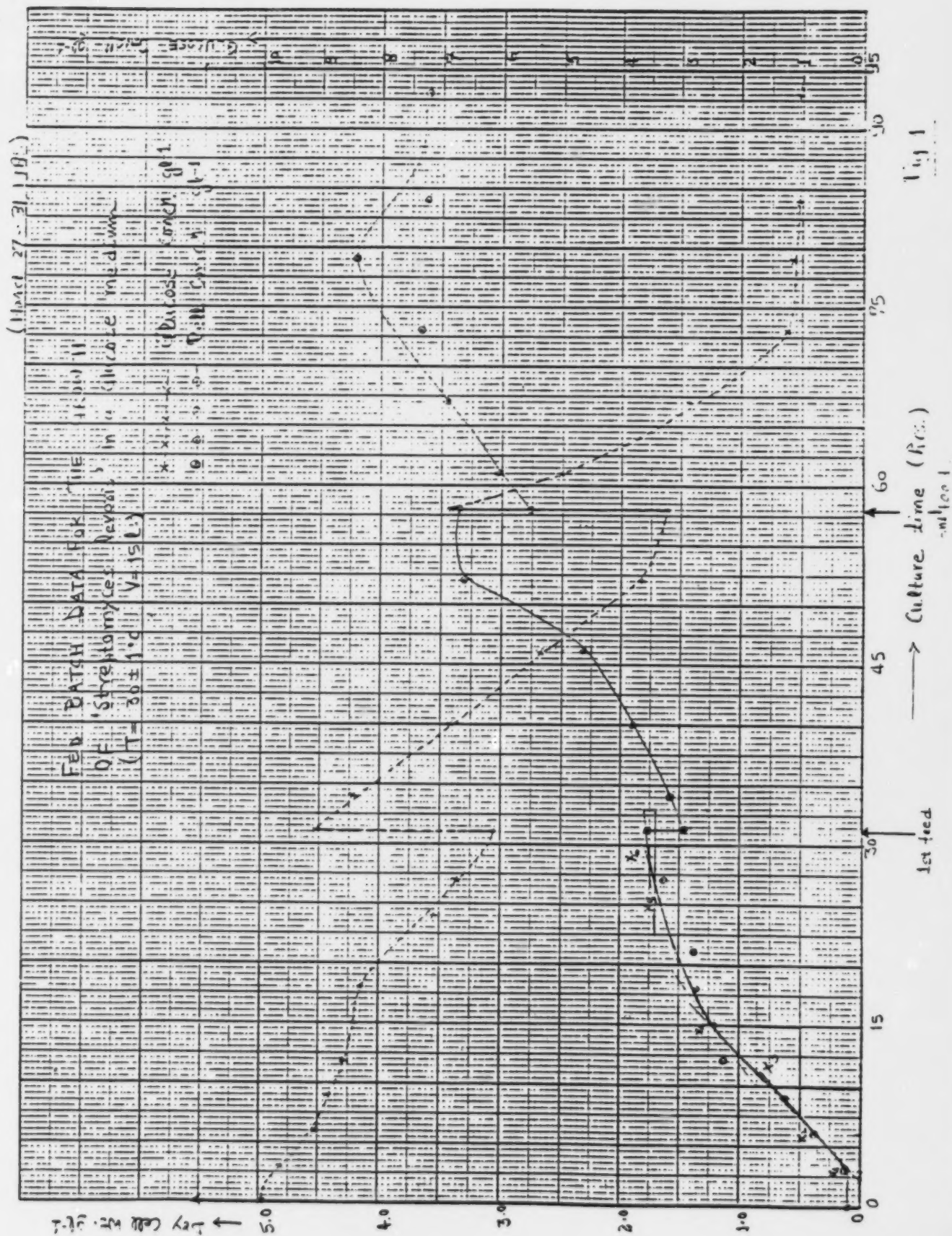
$$Y_{x/s} \Big|_{2nd} = 0.329$$

For growth after 2nd feed

$$\Delta x = 4.215 - 2.7575 = 1.4575$$

$$\Delta S = 6.84 - 1.179 = 5.661$$

$$Y_{x/s} \Big|_{3rd} = 0.257$$



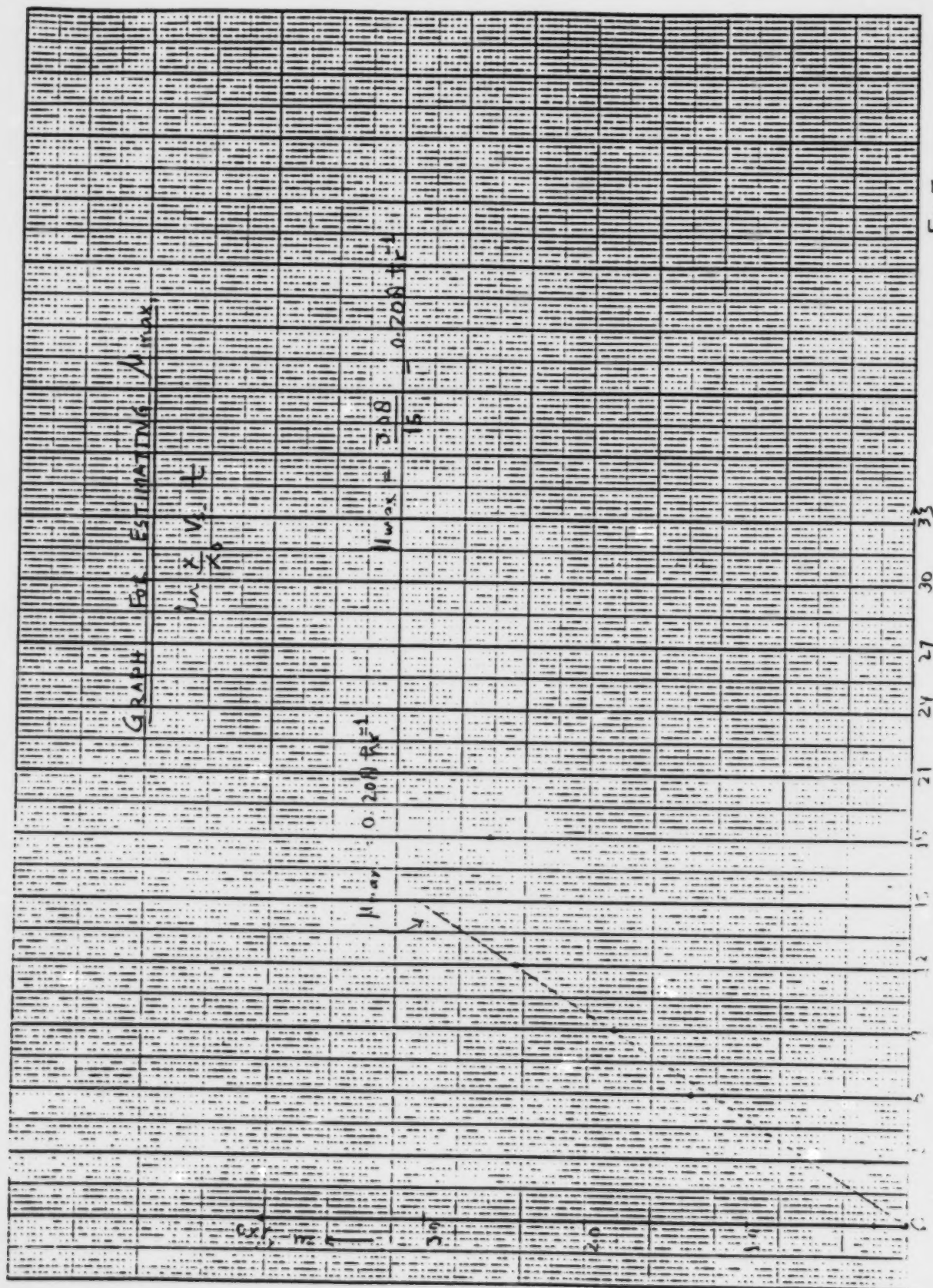
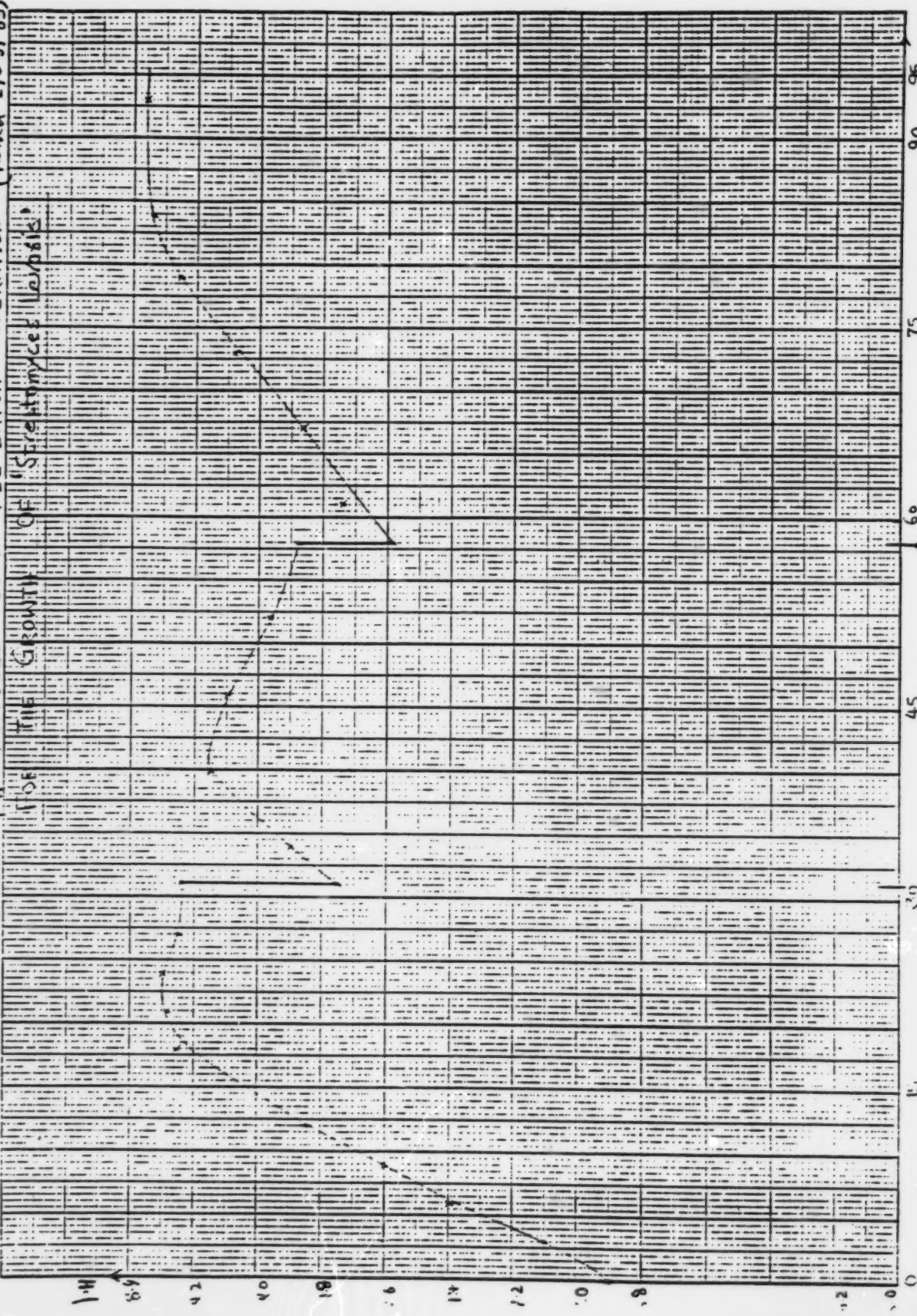


Fig 3

→ t (P.O.S.)

pH - PROFILES IN A FED BATCH OPERATION (March 27-31'85)



→ Culture time (h.) Fig 2

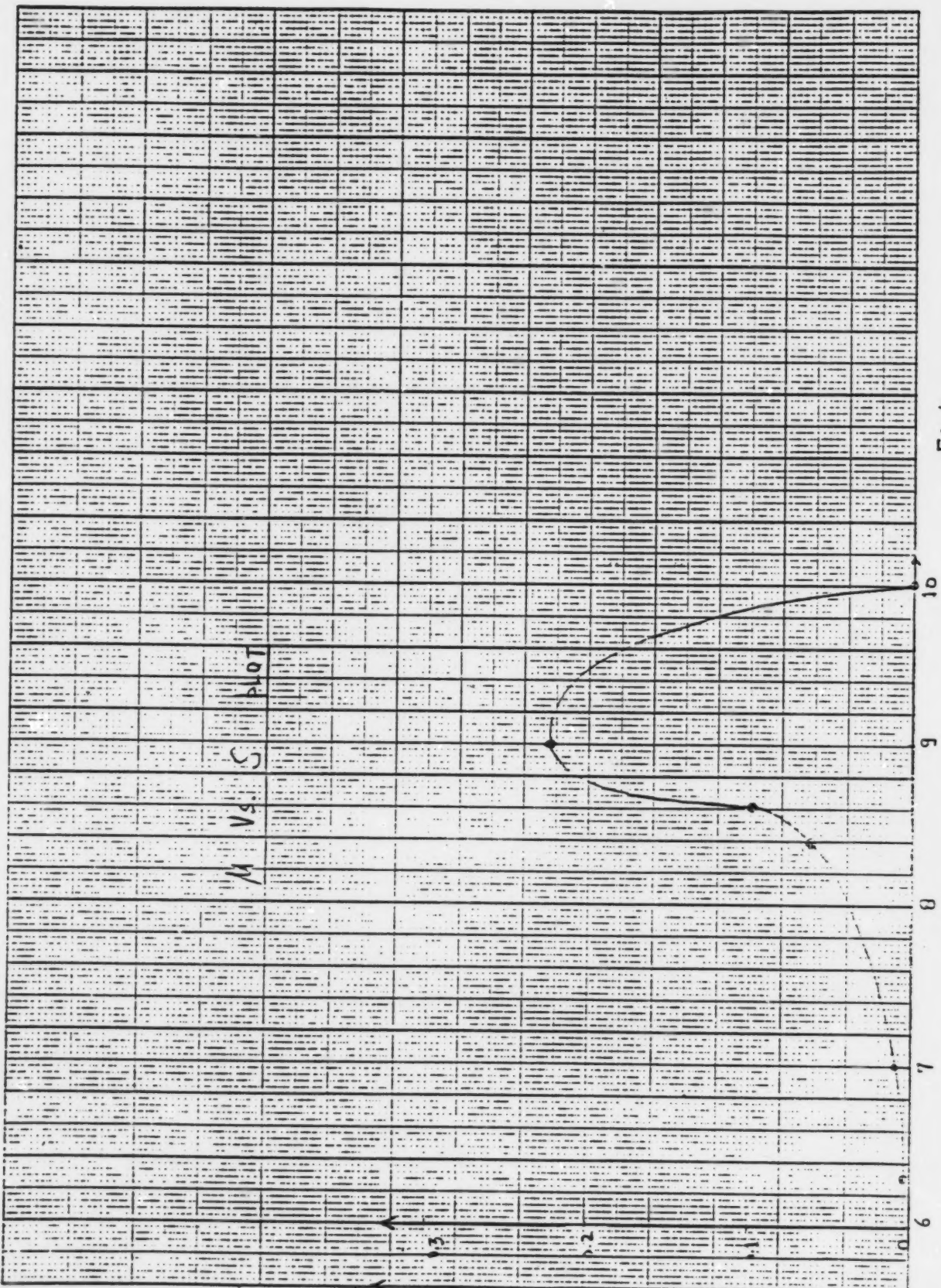


Fig 4

→ S (g l^{-1})

(0.2K) 7/1

APPENDIX B-2

URANIUM ADSORPTION - EXPERIMENTAL DATA

TEMPERATURE EFFECTS
4°C AND 35°C
INACTIVATED VS. VIABLE

URANIUM BIOADSORPTION

35

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 1.37 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	23.8	16.9	17.2	21.8
0.50	21.8	18.4	14.6	23.7
1.00	16.0	22.6	14.1	24.0
2.00	14.0	24.0	11.0	26.3

URANIUM BIOADSORPTION

31

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
s = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 1.37 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	13.9	30.3	17.5	27.7
0.50	10.4	32.8	15.5	29.1
1.00	7.9	34.7	9.5	33.5
2.00	-	-	9.5	33.5

URANIUM BIOADSORPTION

30

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Viable

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
s = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 1.37 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	31.4	17.5	30.3	18.3
0.50	26.3	21.1	24.7	22.4
1.00	18.7	26.8	19.6	26.1
2.00	16.0	28.8	17.0	28.0

URANIUM BIOADSORPTION

34

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Viable

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 1.37 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	38.4	6.28	41.2	4.23
0.50	37.5	6.93	36.3	7.81
1.00	35.8	8.18	32.9	10.30
2.00	30.9	11.80	25.0	16.10

URANIUM BIOADSORPTION

39

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated (Aged)

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 1.58 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	33.0	8.86	41.9	3.23
0.50	28.5	11.70	37.6	5.95
1.00	22.9	15.30	35.0	7.95
2.00	16.3	19.4	29.7	11.0

URANIUM BIOADSORPTION

27

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated (aged)

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
s = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 1.58 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	17.3	24.1	20.6	22.0
0.50	13.2	26.7	13.5	26.5
1.00	7.7	30.2	8.9	29.4
2.00	5.1	31.8	7.1	30.6

URANIUM BIOADSORPTION

38

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable (aged)

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 1.58 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	30.8	10.3	30.1	10.7
0.50	15.5	19.9	23.0	15.2
1.00	-	-	16.5	19.3
2.00	-	-	15.6	19.9

URANIUM BIOADSORPTION

26

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable (aged)

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L

S = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 1.58 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	7.4	30.4	6.3	31.1
0.50	6.1	31.2	6.4	31.0
1.00	6.1	31.2	5.5	31.6
2.00	6.0	31.3	5.4	31.7

URANIUM BIOADSORPTION

1

BIOMASS TYPE : Mixed Culture

CONDITION : Inactivated

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 0.88 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	33.4	15.5	39.1	9.0
0.50	24.2	25.9	32.2	16.8
1.00	17.9	33.1	26.0	23.9
2.00	12.6	39.1	19.2	31.6

URANIUM BIOADSORPTION

29

BIOMASS TYPE : Mixed Culture

CONDITION : Inactivated

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
s = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 0.88 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	22.5	37.4	25.5	34.0
0.50	14.6	46.4	14.8	46.1
1.00	12.8	48.4	12.2	49.0
2.00	8.0	53.9	11.0	50.5

URANIUM BIOADSORPTION

40

BIOMASS TYPE : Mixed Culture

CONDITION : Viable

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 0.88 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	19.0	31.8	31.7	17.4
0.50	30.1	19.2	30.2	19.1
1.00	22.4	28.0	24.3	25.8
2.00	18.4	32.5	15.5	35.8

URANIUM BIOADSORPTION

28

BIOMASS TYPE : Mixed Culture

CONDITION : Viable

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
 $s = 3.2 \text{ mg/L (8)}$

BIOMASS CONCENTRATION : 0.88 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	19.1	41.3	15.3	45.6
0.50	12.2	49.1	12.9	48.3
1.00	6.1	56.0	6.2	55.9
2.00	3.5	59.0	4.9	57.4

URANIUM BIOADSORPTION

41

BIOMASS TYPE : Chlorella vulgaris

CONDITION : Viable

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 1.105 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	21.6	25.0	16.6	30.0
0.50	23.9	22.8	15.7	30.8
1.00	19.6	20.7	14.0	32.5
2.00	16.3	30.3	13.9	32.6

URANIUM BIOADSORPTION

32

BIOMASS TYPE : Chlorella vulgaris

CONDITION : Viable

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
s = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 1.015 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	26.6	28.4	25.1	30.0
0.50	25.8	29.2	24.5	30.4
1.00	23.9	31.0	24.9	30.0
2.00	24.1	30.8	25.1	29.9

URANIUM BIOADSORPTION

42

BIOMASS TYPE : Chlorella vulgaris

CONDITION : Inactivated

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)BIOMASS CONCENTRATION : 1.015 g/L
0.769 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	24.0	22.7	24.8	28.9
0.50	24.5	22.2	22.5	31.9
1.00	15.4	31.1	20.3	34.7
2.00	15.7	30.8	20.9	33.9

URANIUM BIOADSORPTION

33

BIOMASS TYPE : Chlorella vulgaris

CONDITION : Inactivated

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
s = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 1.015 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	34.8	20.3	34.0	21.1
0.50	33.6	21.5	32.1	23.0
1.00	29.5	25.5	26.8	28.2
2.00	25.8	29.2	24.1	30.8

URANIUM BIOADSORPTION

37

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Inactivated

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 1.455 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	16.0	21.3	15.5	21.7
0.50	13.1	23.3	14.1	22.6
1.00	10.8	24.9	11.6	24.3
2.00	11.0	24.7	10.3	25.2

URANIUM BIOADSORPTION

25

BIOMASS TYPE : Saccharomyces cerevisie

CONDITION : Inactivated

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
s = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 1.455 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	7.1	33.2	6.7	33.5
0.50	2.2	35.6	1.9	36.8
1.00	2.7	36.2	2.9	36.1
2.00	1.6	37.0	1.6	37.0

URANIUM BIOADSORPTION

36

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Viable

TEST TEMPERATURE : 4 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 63.1 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 47.0 mg/L
s = 4.59 mg/L (8)

BIOMASS CONCENTRATION : 1.455 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	13.1	23.3	6.3	28.0
0.50	12.4	23.8	8.8	26.3
1.00	6.2	28.0	6.7	27.7
2.00	3.4	30.0	8.5	26.5

URANIUM BIOADSORPTION

24

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Viable

TEST TEMPERATURE : 35 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 67.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 55.4 mg/L
s = 3.2 mg/L (8)

BIOMASS CONCENTRATION : 1.455 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	6.0	34.0	3.4	35.7
0.50	5.5	34.3	2.3	36.5
1.00	6.1	33.9	2.7	36.2
2.00	5.3	34.4	4.2	35.2

pH EFFECTS, 20°C
INACTIVATED VS. VIABLE

URANIUM BIOADSORPTION

22

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
s = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 1.08 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	212	16.02	224	4.91
0.50	208	19.72	217	11.39
1.00	199	28.06	216	12.31
2.00	212	16.02	219	9.54

URANIUM BIOADSORPTION

23

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
s = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 1.31 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	225	3.28	217	9.39
0.50	227	1.76	205	18.55
1.00	228	0.99	221	6.34
2.00	226	2.52	219	7.86

URANIUM BIOADSORPTION

21

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
s = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 0.585 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	215	24.44	220	15.90
0.50	211	31.28	212	29.57
1.00	209	34.70	216	22.74
2.00	204	43.25	206	39.83

URANIUM BIOADSORPTION

21

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
s = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 0.585 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	215	24.44	220	15.90
0.50	211	31.28	212	29.57
1.00	209	34.70	216	22.74
2.00	204	43.25	206	39.83

URANIUM BIOADSORPTION

20

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
s = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 0.585 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	228	2.22	208	36.41
0.50	222	12.48	221	14.19
1.00	225	7.35	221	14.19
2.00	216	22.74	224	9.06

URANIUM BIOADSORPTION

19

BIOMASS TYPE : Mixed Culture

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
s = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 0.846 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	214	18.09	223	7.45
0.50	216	15.72	218	13.36
1.00	205	28.72	225	5.08
2.00	212	20.45	225	5.08

URANIUM BIOADSORPTION

18

BIOMASS TYPE : Mixed Culture

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
S = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 0.846 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	222	8.63	228	1.54
0.50	209	24.00	219	12.17
1.00	213	19.27	213	19.27
2.00	197	38.18	215	16.90

URANIUM BIOADSORPTION

16

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
s = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 1.455 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	222	5.02	204	17.39
0.50	221	5.70	211	12.58
1.00	212	11.89	210	13.26
2.00	214	10.52	210	13.26

URANIUM BIOADSORPTION

17

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor (as rec'd)

INITIAL pH : 2.0

FINAL pH : 2.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 244 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 229.3 mg/L
s = 5.2 mg/L (6)

BIOMASS CONCENTRATION : 1.455 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	203	18.08	214	10.52
0.50	223	4.33	214	10.52
1.00	229	0.21	221	5.70
2.00	224	3.64	213	11.20

URANIUM BIOADSORPTION

55

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 184.0 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 177.0 mg/L
= 6.46 mg/L (8)

BIOMASS CONCENTRATION : 1.425 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	163	9.8	157	14.0
0.50	162	10.5	151	18.3
1.00	160	11.9	106	40.8
2.00	153	16.8	155	15.4

URANIUM BIOADSORPTION

58

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 184.0 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 177.0 mg/L
s = 6.46 mg/L (8)

BIOMASS CONCENTRATION : 1.23 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	160	13.8		
0.50	155	17.9		
1.00	154	18.7		
2.00	149	22.8		

URANIUM BIOADSORPTION

57

BIOMASS TYPE : Mixed Culture

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 184.0 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 177.0 mg/L
s = 6.46 mg/L (8)

BIOMASS CONCENTRATION : 1.02 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	144	32.5	152	24.5
0.50	150	26.5	156	20.6
1.00	136	40.2	147	29.4
2.00	141	35.8	148	28.4

URANIUM BIOADSORPTION

54

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 184.0 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 177.0 mg/L
s = 6.46 mg/L (8)

BIOMASS CONCENTRATION : 1.415 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	141	25.4	149	19.8
0.50	138	27.6	143	24.0
1.00	136	29.0	139	26.9
2.00	133	31.1	-	-

URANIUM BIOADSORPTION

BIOMASS TYPE : Rhizopus oligosporus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 184.0 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 177.0 mg/L
s = 6.46 mg/L (8)

BIOMASS CONCENTRATION : 2.14 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	156	9.81		
0.50	134	20.1		
1.00	117	28.0		
2.00	102	35.1		

URANIUM BIOADSORPTION

56

BIOMASS TYPE : Chaetomium cellulolyticum

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 184.0 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 177.0 mg/L
s = 6.46 mg/L (8)

BIOMASS CONCENTRATION : 5 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	116	12.2	131	9.2
0.50	122	11.0	124	10.6
1.00	114	12.6	109	13.6
2.00	110	13.4	109	13.6

URANIUM BIOADSORPTION

3

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.0

URANIUM CONCENTRATION (STOCK SOLUTION) : 42.6 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 24.86 mg/L
s = 1.87 mg/L (8)

BIOMASS CONCENTRATION : 1.08 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	13.2	10.8	15.6	8.57
0.50	12.0	11.91	13.3	10.70
1.00	9.0	14.69	9.3	14.41
2.00	7.3	16.26	6.0	17.46

URANIUM BIOADSORPTION

5

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.9

FINAL pH : 4.3

URANIUM CONCENTRATION (STOCK SOLUTION) : 42.6 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 24.86 mg/L
s = 1.87 mg/L (8)

BIOMASS CONCENTRATION : 1.31 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	26.5	-	17.4	5.69
0.50	17.5	5.62	16.3	6.53
1.00	18.9	4.55	9.3	11.80
2.00	14.2	8.06	7.7	13.10

URANIUM BIOADSORPTION

9

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.3

URANIUM CONCENTRATION (STOCK SOLUTION) : 45.3 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 33.00 mg/L
s = 5.51 mg/L (7)

BIOMASS CONCENTRATION : 0.585 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	5.7	46.67	5.9	46.32
0.50	4.7	48.38	4.7	48.38
1.00	3.3	50.77	3.5	50.43
2.00	3.0	51.28	3.4	50.60

URANIUM BIOADSORPTION

8

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.2

URANIUM CONCENTRATION (STOCK SOLUTION) : 45.3 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 33.00 mg/L
s = 5.51 mg/L (7)

BIOMASS CONCENTRATION : 0.585 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	13.2	33.85	11.5	36.75
0.50	11.8	36.24	9.8	39.66
1.00	10.5	38.46	8.9	41.20
2.00	8.7	41.54	7.9	42.91

URANIUM BIOADSORPTION

7

BIOMASS TYPE : Mixed Culture

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.6

URANIUM CONCENTRATION (STOCK SOLUTION) : 45.3 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 33.00 mg/L
s = 5.51 mg/L (8)

BIOMASS CONCENTRATION : 0.846 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	21.2	13.9	18.2	17.5
0.50	18.5	17.1	13.0	23.6
1.00	11.7	25.2	10.8	26.2
2.00	6.5	31.3	5.8	32.1

URANIUM BIOADSORPTION

6

BIOMASS TYPE : Mixed Culture

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.7

URANIUM CONCENTRATION (STOCK SOLUTION) : 45.3 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 33.00 mg/L
s = 5.51 mg/L (7)

BIOMASS CONCENTRATION : 0.846 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	24.5	4.04	24.7	9.81
0.50	17.4	12.4	17.7	18.1
1.00	11.7	25.2	8.7	28.7
2.00	7.1	30.6	5.6	32.4

URANIUM BIOADSORPTION

11

BIOMASS TYPE : Chlorella vulgaris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.6

URANIUM CONCENTRATION (STOCK SOLUTION) : 74.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 65.3 mg/L
s = 6.71 mg/L (8)

BIOMASS CONCENTRATION : 1.095 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	46.9	16.8	42.0	21.3
0.50	44.7	18.8	40.2	22.9
1.00	45.5	18.1	39.5	23.6
2.00	43.6	19.8	39.7	23.4

URANIUM BIOADSORPTION

10

BIOMASS TYPE : Chlorella vulgaris

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.6

URANIUM CONCENTRATION (STOCK SOLUTION) : 74.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 65.3 mg/L

s = 6.71 mg/L (8)

BIOMASS CONCENTRATION : 1.095 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	32.6	29.9	35.9	25.8
0.50	31.4	31.0	34.6	28.0
1.00	31.0	31.3	35.8	27.7
2.00	36.4	26.4	37.8	25.1

URANIUM BIOADSORPTION

4

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.3

URANIUM CONCENTRATION (STOCK SOLUTION) : 42.6 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 24.86 mg/L
s = 1.87 mg/L (8)

BIOMASS CONCENTRATION : 1.455 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	4.2	14.30	4.5	14.09
0.50	4.3	14.23	3.6	14.71
1.00	3.2	14.99	3.4	14.85
2.00	2.6	15.40	2.4	15.54

URANIUM BIOADSORPTION

2

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH : 4.2

URANIUM CONCENTRATION (STOCK SOLUTION) : 42.6 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 24.86 mg/L
s = 1.87 mg/L (8)

BIOMASS CONCENTRATION : 1.455 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	4.2	14.30	4.8	13.88
0.50	3.1	15.06	1.5	16.13
1.00	3.0	15.13	1.8	15.96
2.00	3.0	15.13	1.6	16.10

URANIUM BIOADSORPTION

13

BIOMASS TYPE : Rhizopus oligosporus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 74.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 65.3 mg/L
s = 6.71 mg/L (8)

BIOMASS CONCENTRATION : 0.765 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	20.5	58.6	23.0	55.3
0.50	18.0	61.8	22.7	55.7
1.00	15.3	65.4	20.3	58.8
2.00	4.6	79.3	16.5	63.8

URANIUM BIOADSORPTION

12

BIOMASS TYPE : Rhizopus oligosporus

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 74.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 65.3 mg/L
s = 6.71 mg/L (8)

BIOMASS CONCENTRATION : 0.765 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	50.4	19.5	52.8	16.3
0.50	42.3	30.1	49.9	20.1
1.00	38.2	35.4	45.1	26.4
2.00	35.9	38.4	41.4	31.2

URANIUM BIOADSORPTION

15

BIOMASS TYPE : Chaetomium cellulolyticum

CONDITION : Inctivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 74.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 65.3 mg/L
s = 6.71 mg/L (8)

BIOMASS CONCENTRATION : 5 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	33.9	6.28	27.7	7.52
0.50	24.7	8.12	25.9	7.88
1.00	24.7	8.12	21.8	8.70
2.00	16.9	9.68	18.3	9.40

URANIUM BIOADSORPTION

14

BIOMASS TYPE : Chaetomium cellulolyticum

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 74.9 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 65.3 mg/L
s = 6.71 mg/L (8)

BIOMASS CONCENTRATION : 5 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.25	8.4	11.4		
0.50	6.3	11.8		
1.00	5.4	12.0		
2.00	4.6	12.1		

URANIUM BIOADSORPTION

46

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ Solution with 0.1 M Na_2SO_4 INITIAL pH : 5.0 (Test #1)
3.9 (Test #2)

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 110 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 103 mg/L
s = 1.41 mg/L (2)

BIOMASS CONCENTRATION : 0.786 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
1	82.2	26.46	85.1	22.77
5	65.3	47.96	81.3	27.61
10	60.8	53.69	-	-
30	70.4	41.48	79.6	29.77

URANIUM BIOADSORPTION

47

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ Solution with 0.1 M Na_2SO_4 INITIAL pH : 3.0 Test #1
2.0 Test #2

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 110 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 103 mg/L
s = 1.41 mg/L (2)

BIOMASS CONCENTRATION : 0.786 g/L

SAMPLING TIME (MIN) Hr	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
1	85.1	22.77	91.8	14.25
5	79.7	29.64	81.3	27.61
10	84.5	23.54	81.9	26.84
30	77.4	32.57	82.9	25.57

CONTAMINANT EFFECTS

Sulphate, Fe^{3+} , Ni^{2+} , Zn^{2+} , Cu^{2+} , Co^{2+}

EFFECT OF FERRIC ION ON URANIUM BIOADSORPTION - INACTIVATED

BIOMASS	pH	INITIAL (U) (mg/L)	FINAL (U) (mg/L)	INITIAL (Fe) (mg/L)	FINAL (Fe) (mg/L)	U(ADSORBED) (mg/g)
<u>Rhizopus</u>						
	2.7	175	132	0	1.0	16
	2.7	190	131	19	5	20
	2.8	175	123	44	4	20
	2.8	182	139	92	9	16
<u>Streptomyces</u>						
	2.8	175	112	0	0.2	36
	2.8	162	127	16	4	22
	2.8	171	145	43	10	15
	2.8	167	158	83	34	5.2
<u>Mixed Culture</u>						
	2.8	175	47	0	87	91
	2.8	162	96	16	93	51
	2.8	179	125	45	90	37
	2.8	175	123	88	88	37

URANIUM BIOADSORPTION

48

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ Solution with 0.1 M Na_2SO_4 Test #1: 15 mg/L Fe^{3+} addedTest #2: 15 mg/L Ni^{2+} added

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 110 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 103 mg/L
s = 1.41 mg/L (2)

BIOMASS CONCENTRATION : 0.786 g/L

SAMPLING TIME (MIN) _{hr}	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
1	93.1	12.60	77.8	32.06
5	89.3	17.43	74.8	35.88
10	92.2	13.74	75.5	34.99
30	86.7	20.74	73.0	38.17

URANIUM BIOADSORPTION

49

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ Solution with 0.1 M Na_2SO_4 Test #1: 15 mg/L Zn^{2+} addedTest #2: 10 mg/L Cu^{2+} added

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 110 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 103 mg/L
s = 1.41 mg/L (2)

BIOMASS CONCENTRATION : 0.786 g/L

SAMPLING TIME (MIN) hr	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
1	83.2	25.19	78.7	30.92
5	72.6	38.68	77.1	32.95
10	74.6	36.13	76.5	33.72
30	79.0	30.53	71.4	40.20

URANIUM BIOADSORPTION

50

BIOMASS TYPE : Streptomyces levoris

CONDITION : Viable

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ Solution with 0.1 M Na_2SO_4 Test #1: 15 mg/L Co^{2+} added

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 110 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 103 mg/L
s = 1.41 mg/L (2)

BIOMASS CONCENTRATION : 0.786 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
1	79.0	30.53		
5	78.7	30.92		
10	76.5	33.72		
30	75.8	34.61		

URANIUM BIOADSORPTION

52

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ SolutionTest #1: 0.0 M SO_4^{2-} Test #2: 0.02 M SO_4^{2-}

INITIAL pH : 4.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 220 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 220 mg/L

BIOMASS CONCENTRATION : 1.58 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.5	46.2	110.0	65.1	98.0
1.0	5.6	135.7	14.6	130.0
2.0	3.4	137.1	8.1	134.1
92.0	1.2	138.5	6.8	134.9

URANIUM BIOADSORPTION

53

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ SolutionTest #1: 0.04 M SO_4^{2-} Test #2: 0.06 M SO_4^{2-}

INITIAL pH : 4.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 220 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 220 mg/L

BIOMASS CONCENTRATION : 1.58 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.5	71.6	93.9	73.5	92.7
1.0	26.7	122.4	24.2	123.9
2.0	17.1	128.4	21.4	125.7
92.0	8.1	134.1	11.8	131.8

URANIUM BIOADSORPTION

51

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ SolutionTest #1: 0.08 M SO_4^{2-} Test #2: 0.10 M SO_4^{2-}

INITIAL pH : 4.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 220 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 220 mg/L

BIOMASS CONCENTRATION : 1.58 g/L

SAMPLING TIME (HR)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
0.5	75.8	91.4	91.1	81.6
1.0	23.3	124.5	22.0	125.3
2.0	18.9	127.3	20.2	126.5
92.0	9.3	133.4	11.5	132.0

APPENDIX B-3

EQUILIBRIUM DATA - EXPERIMENTAL RESULTS

pH EFFECTS - 5, 4, AND 3.5
INACTIVATED AND PHYSIOLOGICAL AGE

URANIUM BIOADSORPTION

44

BIOMASS TYPE : Test #1: Rhizopus arrhizus
Test #2: Streptomyces levoris (aged)

CONDITION : Inactivated (Test #1)
Viable (Test #2) TEST TEMPERATURE : °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 127.5 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 127.5 mg/L
s = 4.95 mg/L ()

BIOMASS CONCENTRATION : 1.37 g/L (Test #1)
1.58 g/L (Test #2)

VOLUME OF SOLUTION (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	25.8	74.2	44.8	52.3
195	50.2	112.8	72.9	69.1
290	66.8	132.9	85.7	79.4
385	79.9	139.0	99.9	69.9

URANIUM BIOADSORPTION

78

EQUILIBRIUM STUDIES

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 118.0 mg/L

BIOMASS CONCENTRATION : 1.38 g/L (dry wt)

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	21	70.29		
195	44	108.08		
290	63	121.78		
385	74	130.25		

URANIUM BIOADSORPTION

43

BIOMASS TYPE : Test #1 Mixed Culture
Test #2 Chlorella vulgaris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 5.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 127.5 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 127.5 mg/L
s = 4.95 mg/L ()

BIOMASS CONCENTRATION : Test #1: 0.88 g/L
Test #2: 1.015 g/L

VOLUME OF SOLUTION (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	47.5	90.8	71.2	55.5
195	72.9	116.6	91.6	70.7
290	94.6	112.2	103.0	72.4
385	104.0	106.8	113.0	57.1

URANIUM BIOADSORPTION

60

EQUILIBRIUM STUDIES

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 4.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 174.3 mg/L
s = 4.51 mg/L (4)

BIOMASS CONCENTRATION : 1.70 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	119	32.6		
195	132	50.3		
290	146	51.3		
385	155	47.6		

URANIUM BIOADSORPTION

62

EQUILIBRIUM STUDIES

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 4.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 174.3 mg/L
s = 5.46 mg/L (4)

BIOMASS CONCENTRATION : 0.95 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	122	55.1		
195	130	93.7		
290	151	76.2		
385	153	92.6		

URANIUM BIOADSORPTION

63

EQUILIBRIUM STUDIES

BIOMASS TYPE : Mixed Culture

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 4.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 174.3 mg/L
S = 4.51 mg/L (4)

BIOMASS CONCENTRATION : 0.83 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	120	65.42		
195	150	60.36		
290	164	40.72		
385	166	43.86		

URANIUM BIOADSORPTION

61

EQUILIBRIUM STUDIES

BIOMASS TYPE : Chlorella vulgaris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 4.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 174.3 mg/L
s = 4.51 mg/L (4)

BIOMASS CONCENTRATION : 1.06 g/L (INITIAL)

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	160	13.5		
195	160	27.0		
290	165	26.8		
385	168	24.7		

URANIUM BIOADSORPTION

66

EQUILIBRIUM STUDIES

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 256 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 243 mg/L
s = 12.99 mg/L (4)

BIOMASS CONCENTRATION : 1.65 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	172	43.0	182	37.0
195	200	53.0	200	53.0
290	222	40.0	224	36.6
385	227	41.4	230	34.1

URANIUM BIOADSORPTION

70

EQUILIBRIUM STUDIES

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ solution with 0.02 M Na_2SO_4

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 194 mg/L

BIOMASS CONCENTRATION : 5.0 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100/10	4.6	32.2		
195 205	26.4	57.7		
290 300	54.0	74.1		
285 395	82.0	82.1		

URANIUM BIOADSORPTION

67

EQUILIBRIUM STUDIES

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 256 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 243 mg/L
s = 12.99 mg/L (4)

BIOMASS CONCENTRATION : 2.5 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	232	13.3	226	20.5
195	242	3.0	226	41.0
290	239	14.7	232	40.5
385	231	56.6	233	49.1

URANIUM BIOADSORPTION

69

EQUILIBRIUM STUDIES

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 256 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 243 mg/L

BIOMASS CONCENTRATION : 0.92 g/L (Test #1)

0.76 g/L (Test #2)

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	170	79.4	199	57.9
195	204	86.6	215	74.7
290	229	50.2	236	31.5
385	231	57.1	232	60.9

URANIUM BIOADSORPTION

71

EQUILIBRIUM STUDIES

BIOMASS TYPE : Streptomyces levoris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ solution with 0.02 M Na_2SO_4

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 194 mg/L

BIOMASS CONCENTRATION : 0.828 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100 110	31	21.7	31	21.7
195 205	40	41.8	37	42.5
290 300	32	65.9	43	62.0
385 395	47	80.1	52	78.6

URANIUM BIOADSORPTION

64

EQUILIBRIUM STUDIES

BIOMASS TYPE : Mixed Culture

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 256 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 243 mg/L
s = 12.99 mg/L (4)

BIOMASS CONCENTRATION : 0.77 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	212	40.5	207	47.1
195	225	47.9	224	50.8
290	233	41.1	229	56.7
385	234	49.2	221	-

URANIUM BIOADSORPTION

68

EQUILIBRIUM STUDIES

BIOMASS TYPE : Chlorella vulgaris

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 256 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 243 mg/L
s = 12.99 mg/L (4)

BIOMASS CONCENTRATION : 1.88 g/L

SAMPE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	212	16.5	218	13.3
195	216	28.8	213	31.8
290	234	14.4	235	18.8
385	237	14.1	231	26.3

EQUILIBRIUM STUDIES

BIOMASS TYPE : Saccharomyces cerevisiae

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 256 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 243 mg/L
s = 12.99 mg/L (4)

BIOMASS CONCENTRATION : 1.80 g/L

SAMPLE VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	187	31.1	164	43.9
195	220	26.5	201	47.7
290	224	32.8	219	42.0
385	232	26.3	218	57.5

APPENDIX B-4

BATCH URANIUM DESORPTION

EXPERIMENTAL DATA

BATCH ADSORPTION/DESORPTION STUDIES

URANIUM BIOADSORPTION

77

EQUILIBRIUM STUDIES

BIOMASS TYPE : Mixed Culture

EQUIVALENT DRY WT: 0.162 g

CONDITION : Inactivated

ADSORPTION MEDIUM: Synthetic $\text{UO}_2(\text{NO}_3)_2$ soln. with 0.02 M Na_2SO_4

ADSORPTION pH: 3.9

DESORPTION MEDIUM : TEST #1: 0.02 N H_2SO_4 (pH = 2.05)
TEST #2: 0.1 M $(\text{NH}_4)_2\text{SO}_4$ (pH = 5.3)
TEST #3: 0.02 M EDTA (disodium salt) pH = 4.7)
TEST #4: 0.1 M NaHCO_3 (pH = 8.2)

TEST #	ADSORPTION			DESORPTION	
	Initial U (mg/L)	Final U (mg/L)	U(ADS) (mg/g)	Final U (mg/L)	U(Desorbed) (mg/g)
1	174	28	104	116	72
2	179	16	112	0.1	0.1
3	161	12	114	19	12
4	172	16	113	163	101

URANIUM BIOADSORPTION

76

EQUILIBRIUM STUDIES

BIOMASS TYPE : Streptomyces levoris Equivalent Dry Wt. 0.184 g

CONDITION : Inactivated

TEST TEMPERATURE : °C

ADSORPTION MEDIUM: Synthetic $\text{UO}_2(\text{NO}_3)_2$ soln. with 0.02 M Na_2SO_4

ADSORPTION pH : 3.9

DESORPTION MEDIUM : TEST #1: 0.02 N H_2SO_4 (pH = 2.05)
TEST #2: 0.1 M $(\text{NH}_4)_2\text{SO}_4$ (pH = 5.3)
TEST #3: 0.02 M EDTA (disodium salt) pH = 4.7)
TEST #4: 0.1 M NaHCO_3 (pH = 8.2)

TEST #	ADSORPTION			DESORPTION	
	Initial U (mg/L)	Final U (mg/L)	U(ADS) (mg/g)	Final U (mg/L)	U(Desorbed) (mg/g)
1	179	39	78	83	42
2	165	48	71	22	11
3	167	31	81	70	35
4	172	40	77	145	73

URANIUM BIOADSORPTION

75

EQUILIBRIUM STUDIES

BIOMASS TYPE : Rhizopus arrhizus

EQUIVALENT DRY WT: 0.306 g

CONDITION : Inactivated

TEST TEMPERATURE : °C

ADSORPTION MEDIUM : Synthetic $\text{UO}_2(\text{NO}_3)_2$ solution with 0.02 M Na_2SO_4

INITIAL pH : 3.5

DESORPTION MEDIUM: TEST #1: 0.02 N H_2SO_4 (pH = 2.05)
TEST #2: 0.1 M $(\text{NH}_4)_2\text{SO}_4$ (pH=5.3)
TEST #3: 0.02 M EDTA (disodium salt, pH = 4.7)
TEST #4: 0.1 M NaHCO_3 (pH=8.2)

TEST #	ADSORPTION			DESORPTION	
	Initial U (mg/L)	Final U (mg/L)	U(ADS) (mg/g)	Final U (mg/L)	U(Desorbed) (mg/g)
1	177	10	62	72	24
2	175	11	61	2	0.7
3	175	12	61	8	2.6
4	172	12	61	147	48

APPENDIX B-5

COLUMN STUDIES

Uranium Bioadsorbtion Column Studies

Biomass Type: Rhizopus arrhizus

Test Temperature: 20°C

Condition: Inactivated

Medium: $\text{UO}_2(\text{NO}_3)_2$ Solution

Initial pH = 3.5

Uranium Concentration (Test Solution) = 188 mg/L

Biomass Concentration: 0.54 g

Loading Data

Volume of Effluent (mL)	pH	Flow Rate (mL/min)	U (ppm)	<u>Total U Absorbed</u>		Fe (ppm)	Zn (ppm)
				(mg)	(mg/g biomass)		
0	4.0	2.9	0*	0	0	<0.1**	0.3**
50	4.25	2.9	96	7.0	13.0	1.6	0.95
100	4.15	2.9	125	10.85	20.1	1.1	0.7
200	4.05	3.1	152	15.75	29.2	0.8	0.45
300	4.0	3.1	165	18.7	34.6	0.7	0.4
400	4.0	3.0	171	20.7	38.3	0.6	0.3
500	4.0	3.0	174	22.25	41.2	0.6	0.2
600	3.95	2.9	176	23.55	43.6	0.5	0.15
700	3.95	2.8					
800	3.95	3.1	178	25.75	47.7	0.3	0.15
900	3.95	3.2					
1000	4.0	2.9	179	27.65	51.2	0.3	0.1
1200	4.0	3.0	183	29.05	53.8	0.3	0.1
1400	4.0	3.0	185	29.85	55.3	0.2	0.1

* assuming linear increase for first sample period

** contaminants in loading solution

Elution Data

0.1 M NaHCO₃, pH 8.2, 0.5 mL/min

Volume of Eluate (mL)	pH	U (ppm)	U Eluted (Total) (mg) (mg/g biomass)	Fe	Zn
5	4.1	128	0.64	1.2	30 0.6
10	6.1	100	1.14	2.1	25 0.5
15	6.7	135	1.82	3.4	15 0.3
20	6.9	308	3.36	6.2	9 0.1
30	7.1	510	8.46	15.7	5 0
40	7.5	550	13.46	25.9	2 0
50	7.7	400	17.96	33.3	1 0
60	7.9	252	20.48	37.9	<1 0
			(69%)		
70	8.1	163	22.11	40.9	<1 0
80	8.1	102	23.13	42.8	<1 0
90	8.1	65	23.78	44.0	<1 0
100	8.1	42*	24.47	45.3	<1 0
120	8.1	18*	24.65	45.6	<1 0
130	8.1	12	24.77	45.9	<1 0
140	8.1	8*	24.85	46.0	<1 0
150	8.1	5	24.90	46.1	<1 0
			(83%)		

* estimated from elution curve

URANIUM BIOADSORPTION

74

COLUMN STUDIES

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM :

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 207 mg/L
s = 8.49 mg/L (8)

BIOMASS CONCENTRATION : 3.02 g/L

ELUENT VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
10	0.6			
20	9.0			
100	30.4			
500	148.0			
1000	180.0			
Desorbition (0.1 M NaHCO ₃ , pH = 8.2)				
10	130.0			
50	667.0			
100	409.0			
Readsorbition				
10	4.8			
20	23.7			
100	93.8			
500	156.0			
Desorbition (0.1 M Na HCO ₃ , pH = 8.2)				
50	355.0			
100	421.0			

COLUMN STUDIES

BIOMASS TYPE : Rhizopus arrhizus

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

FLOW: TEST #1 1.5 mL/min
(0.85 mL/cm² min)

INITIAL pH : 3.5

TEST #2 4.0 mL/min
(2.26 mL/cm² min)

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : 185 mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 182.67 mg/L
s = 7.77 mg/L (3)

BIOMASS CONCENTRATION : 1.43 g/L

SAMPLING TIME (min)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
10	1.8	1.9	127	1.56
20	8.3	1.83	133	1.39
30	16.2	1.75	145	1.05
45	30.0	2.41	156	1.12
60	49.8	2.09	165	0.74
90	83.8	3.11	170	1.06
120	110.0	2.29	170	1.06
150	140.0	1.34	177	0.48
180	162.0	0.65	-	
210	170.0	0.40	-	
	TOTAL	17.77		8.46

URANIUM BIOADSORPTION

73

COLUMN STUDIES

BIOMASS TYPE : Streptomyces levoris (Test #1)
Mixed Culture - Immobilized (Test #2)

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM :

FLOW: 2.2 mL/min (Test #1)

2.0 mL/min (Test #2)

INITIAL pH : 3.5

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 207 mg/L
s = 8.49 mg/L (3)

BIOMASS CONCENTRATION : 1.84 g (TEST #1)
1.56 g (TEST #2)

ELUENT VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
10	0.6		1.0	
20	12.8		8.3	
100	118.0		88.6	
500	147.0		166.0	
1000	181.0		184.0	
Desorption (0.1 M Na HCO ₃ pH = 8.2				
10	107.0		931.0	
50	666.0		422.0	
100	275.0		177.0	
Readsorbition				
10	7.0		2.6	
20	13.4		23.7	
100	117.0		42.6	
500	195.0		102.0	
Desorbition (0.1 M Na HCO ₃ pH = 8.2				
50	397.0		586.0	
100	595.0		275.0	

Column Study

BIOMASS TYPE : Rhizopus arrhizus (Test #1) immobilized
Streptomyces levoris (Test #2) immobilized

CONDITION : Inactivated

TEST TEMPERATURE : 20 °C

MEDIUM : Process Liquor

Flow: 5 mL/min
 (2.84 mL/cm².m)

INITIAL pH : 4.0

FINAL pH :

URANIUM CONCENTRATION (STOCK SOLUTION) : mg/L

URANIUM CONCENTRATION (TEST SOLUTION) : 155 mg/L

BIOMASS CONCENTRATION : 1.37 g (Test #1)
 1.58 g (Test #2)

ELUENT VOLUME (mL)	TEST #1		TEST #2	
	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)	URANIUM CONC (mg/L)	URANIUM ADS. (mg/g)
100	72.8	6.0	51.9	6.53
200	100.0	4.01	86.5	4.34
300	111.0	3.21	110.0	2.85
400	127.0	2.04	115.0	2.53
500	126.0	2.12	127.0	1.77
600	133.0	1.61	131.0	1.52
700	141.0	1.02	140.0	0.95
800	142.0	0.95	139.0	1.01
900	142.0	0.95	141.0	0.89
1000	144.0	1.80	145.0	0.63
1200	144.0	1.61	151.0	0.51
1400	151.0	0.58	149.0	0.76
1600	149.0	0.88	154.0	0.13
1800	151.0	0.58	150.0	0.63
2000	153.0	0.29	153.0	0.25
2200	151.0	0.88	150.0	0.63
2400	151.0	0.88	154.0	0.13
	TOTAL 28.41		TOTAL 26.06	

EVALUATION OF BIOMASS FOR URANIUM RECOVERY FROM PROCESS STREAMS
STAGE III - BIOMASS EVALUATION

FINAL REPORT

BY

SENE CONSULTANTS LIMITED

June 1985

Work on this project was conducted under the auspices of the Canada Centre for Mineral and Energy Technology, Energy, Mines and Resources, Canada.

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ABSTRACT

Five biomasses Rhizopus arrhizus, Streptomyces levoris, a mixed culture sewage sludge, Saccharomyces cerevisiae and Chorella vulgaris were evaluated for their potential for utilization in a uranium recovery process. All biomasses displayed good ability to adsorb uranium however the physical properties of the biomasses, their cost of production and amenability to process applications are quite different.

Rhizopus arrhizus proved to be the superior organism tested. This organism has good mechanical properties, is readily dewatered, and was of the least expensive to produce. The second choice was the mixed culture and this is primarily due to the low cost of purchase. The application potential for the other organisms tested, especially the algae, is very low.

There are technical issues but none that could not be resolved. The major hurdle is likely to be economics. Production costs were estimated to be \$30-35/kg of U_3O_8 produced.

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1.0 INTRODUCTION

1.1 Overview

Numerous micro-organisms have an excellent ability for concentrating metals through bioadsorption. The Stage II laboratory investigation for this study identified 7 organisms with excellent adsorption capabilities and concluded that many other organisms could potentially be equally as efficient as those tested. It also became apparent that the micro-organisms tested were not selectively adsorbing uranium and that competition with other cations for the available adsorption sites was likely occurring. However the basic conclusion drawn from the Stage II work was that bioadsorption of uranium from process solutions is a significant process with potential for full scale application.

1.2 Objectives

The objectives of this report is to evaluate the Stage II laboratory data along with other relevant information with the intent of comparing the various biomasses on their

- cost of production
- adaptability to convention contact equipment
- process applicability
- potential economic hurdles
- major technical problems

1.3 Study Team

The study team included members from SENES Consultants Limited, A.H. Ross and Associates and the Institute for Biotechnology.

SENES Consultants Limited managed the projects and were the prime

contributors. A.H. Ross and Associates provided input to the adaptation of biomass to convention process technology and the Institute for Biotechnology provide general guidance on biomass production.

2.0 BIOMASS PRODUCTION

2.1 General

The key to the development of any successful process application will be the production of a low cost biomass. The major components of the biomass cost are the capital cost for the manufacturing facilities and the operating cost for production. In this section a review of the potential capital and operating costs for biomass production are provided.

2.2 Capital Costs

For the production of the biomass, a standard 1 ton/day flow sheet has been developed. The flowsheet provides for

- . stainless steel fermentor sized according to growth rates and yields obtained in this study.
- . vacuum filter sized according to the relative ease of filtration (108 kg/m².d for Rhizopus and 65 kg/m².d for all others) to obtain 20% dry weight biomass in the cake
- . rotary drum drier sufficient to reduce water content from 80% to 5%.
- . storage tank
- . sterilizer to inactivate biomass
- . heat exchanger to minimize heat loss

The major process design parameters are summarized in Table 2.1 and the capital costs for the major process equipment are summarized in Table 2.2.

The total capital breakdown for the biomass production are summarized in Table 2.3. These total costs include

- . major production equipment (from Table 2.1)
- . allowance of 80% for installation

Table 2.1

BIOLOGICAL AND MAJOR PROCESS DESIGN PARAMETERS
1 TON/DAY (DRY WEIGHT BASIS)

	<u>R. arrhizus</u>	<u>S. levoris</u>	<u>S. cerevisiae</u>	<u>C. vulgaris</u>
Design Specific Growth Rate (hr^{-1})	0.13	0.15	0.16	0.02
Biomass Growth Yield (g/g)	0.45	0.33	0.42	0.32
Biomass Conc. in Fermentor Effluent (g/L)	9.0	6.6	8.4	3.0
Flow (L/day)	111,111	151,515	119,058	333,000
Fermentation Volume (L)	35,612	42,087	31,002	694,443
Design Fermentation Volume (L) (1.3xtheoretical)	46,000	55,000	40,000	900,000

Table 2.2

MAJOR EQUIPMENT

	<u>R. arrhizus</u>	<u>S. levoris</u>	<u>S. cerevisiae</u>	<u>C. vulgaris</u>
Permentor (stainless steel air lift)	64,500	73,100	58,500	517,000
Vacuum filter	47,000	67,200	67,200	67,200
Rotary drum dryer	97,600	97,600	97,600	97,600
Storage tank (mild steel, 50,000 L)	22,800	22,800	22,000	22,800
Sterilizer, direct steam injection	32,000	32,000	32,000	32,000
Heat exchange	<u>21,500</u> \$285,400	<u>21,500</u> \$313,700	<u>21,500</u> \$299,600	<u>21,000</u> \$758,100

Table 2.3

CAPITAL COST ESTIMATIONS*

<u>Item</u>	<u>BIOMASS</u>			
	<u>R. arrhizus</u>	<u>S. levoris</u>	<u>S. cerevisiae</u>	<u>C. vulgaris</u>
1) Delivered Equip. Cost	285,400	31,700	299,600	758,100
2) Installed Equipment	513,700	564,700	539,300	1,137,100
3) Process Piping	102,700	102,900	102,900	113,700
4) Instrumentation	60,000	60,000	60,000	80,000
5) Buildings and Site	205,500	225,900	215,700	454,900
6) Auxiliaries (pums etc).	308,200	338,800	323,600	680,000
7) Utilities	15,400	16,900	16,200	34,100
8) Total Physical Plant	1,205,500	1,309,200	1,257,700	2,499,800
9) Engineering	361,700	392,800	377,300	749,900
10) Contingency	482,200	523,700	503,100	999,9000
11) Size Factor	120,600	130,900	125,800	250,000
12) Total Plant Cost	<u>2,170,000</u>	<u>2,356,600</u>	<u>2,263,900</u>	<u>4,499,600</u>

* Cost factors from Peters, M.S. and V.D. Timmerhaus, Plant Design and Economics for Chemical Engineers, McGraw hill, 1968.

- . process piping 20% of installed cost
- . instrumentation (lump sum)
- . buildings and land purchase (40% of installed cost)
- . auxilliary pumps valves tankage etc. (60% of installed cost)
- . utilities (3% of plant cost)
- . contingency (40% of plant cost)
- . size factor adjustment (10% of plant cost)

These costing components and factors have been obtained from Peters and Timmerhaus (1968). As shown on Table 2.3 the capital costs range from 2,170,000 for Rhizopus arrhizus to 4,499,600 for Chlorella vulgaris.

2.3 Operating Costs

The major components of the operating costs are media purchase and labour costs. Minor costs for maintenance and utilities have also been included. A summary of the costs is provided in Table 2.4.

For the operation of the biomass production plant a staff of 5 operators and one administration personnel would be required. Total costs including overhead and payroll purchases of \$40,000/person have been provided (i.e. \$240,000).

Media costs are highly variable and have been calculated using the formulation for the media specified in the Stage II report and current chemical purchase costs. As shown on Table 2.4, both the Rhizopus arrhizus and Saccharomyces cerevisiae have a moderately priced media and Streptomyces levoris and Chlorella vulgaris a complex and expensive media.

A utilities allowance of \$50,000 for power, water and sewer was

Table 2.4

OPERATING COSTS - 1 ton/d BIOMASS PRODUCTION

	<u>R. arrhizus</u>	<u>S. levoris</u>	<u>S. cerevisiae</u>	<u>C. vulgaris</u>
Labour (6 persons)	240,000	240,000	240,000	240,000
Utilities (allow)	50,000	50,000	50,000	50,000
Media Cost	510,000	880,000	405,000	988,000
Maintenance (5% of plant cost)	<u>60,000</u>	<u>65,000</u>	<u>65,000</u>	<u>122,000</u>
Total Annual Cost (\$)	860,000	1,235,000	758,000	1,400,000
Biomass Produce (kg/a)	<u>330,000</u>	<u>330,000</u>	<u>330,000</u>	<u>330,000</u>
Total Operating Cost\$/kg	2.61	3.74	2.30	4.24

provided.

A maintenance allowance of 5 percent of the plant cost was provided.

For the 1 ton/day plant, the operating cost range from \$2.30 to \$4.24/kg of biomass produced. This assumes that all materials are purchased. These costs could be reduced by approximately 1.00/kg for R. arrhizus if waste materials could be used as the carbon source for the media. In addition the S. cerevisiae cost could potentially be reduced by about \$1.00/kg if spent brewers yeast was available at no cost.

For a mixed culture, sewage sludge should be available at no cost. Therefore it would only be necessary to dewater, dry and inactivate the culture. The expected operating cost would be \$1.00/kg.

2.4 Total Production Costs

The total biomass production costs will depend upon numerous factors including taxes, write-offs, and miscellaneous allowances. For this analysis it has been conservatively assumed.

- . a plant life of 15 years with no residual value.
- . an operating profit of 15% on the capital expenditure
- . all money is borrowed at 12%.

The overall cost summary is provided in Table 2.5. The total production costs are \$4.50/kg for R. arrhizus, \$5.80/kg for S. levoris, \$4.30 for S. cerevisiae and \$8.25 /kg for C. vulgaris. Costs for R. arrhizus and S. cerevisiae may be reduced by approximately \$1.00/kg if waste materials were available.

Table 2.5

OVERALL COST SUMMARY

Capital Cost	2,170,000	2,356,600	2,263,900	4,499,600
15 yr amortization at 12% interest \$/a	308,000	334,000	321,000	634,000
Profit 15%	<u>326,000</u>	<u>354,000</u>	<u>340,000</u>	<u>675,000</u>
Total Annual Cost (capital)	634,000	688,000	661,000	1,329,000
Total operating cost/a	<u>860,000</u>	<u>1,235,000</u>	<u>748,000</u>	<u>1,400,000</u>
Total Cost/a	1,494,000	1,923,000	1,419,000	2,729,000
Biomass produced kg	<u>330,000</u>	<u>330,000</u>	<u>330,000</u>	<u>330,000</u>
say	4.53	5.83	4.30	8.27
	\$4.50 ¹ /kg	\$5.80/kg	\$4.30 ² /kg	\$8.25/kg

Notes

1. Could be reduced by \$1.00/kg if waste products used for media
2. Could be reduced by approximately \$1.00/kg if waste brewers yeast was available.

Mixed culture could also be received as sewage sludge, dewatered dried and inactivated. The total capital and operating costs for this system would be approximately \$3.00/kg using the same cost factors described previously.



3.0 POTENTIAL PROCESS APPLICATIONS

3.1 General

Biomasses are not likely to replace conventional uranium concentration/purification processes such as ion exchange or solvent extraction. These latter processes are highly efficient, well developed and very cost effective in uranium mill applications. However these processes are somewhat sophisticated and required rigorous operator attention.

Biomass applications are likely to be directed at low grade solutions where minimal process control is possible. In addition where biomass costs are low one time use applications are certainly a possibility. Perhaps the most likely applications deal with recovery of uranium from waste water or bioleach solutions. The primary examples would be

- . acid mine waters
- . acid tailings runoff
- . underground leaching solutions

A discussion of the above potential applications follows.

3.2 Acid Mine Water

A typical acid mine water was described in Chapter 6.1 of the Stage II report, as having a uranium content of 127 mg/L and a pH of 2.7. At this pH and uranium level most any biomass would have an effective net adsorbtion capacity of at least 20 mg U/g and as such 43 kg of biomass would be required per kg of uranium (U_3O_8) recovered. Biomass purchase cost ranges from nil for waste biomass such as sewage sludge or brewery waste to several dollars per kg. Obviously if biomass could be obtained at no cost, one time utilization and disposal of the biomass is possible if

minimal alteration to the biomass as received is required before use. However, if biomass is purchased, it must be capable of repetitive use.

For the one time use application, we foresee a material such as an inactivated thickened sewage sludge applied to the inlet of underground sumps in the mine and allowed to contact with mine water solutions. Because of the long contact times, biomass may reach substantially higher bioadsorption levels. Mine water for use in underground leaching would be recycled from the sumps. As the sumps become full, the fluid like biomass would be pumped to the surface with the mine water (at about 1% solids). Residual biomass would be mucked out with the sump sludges during routine sump clean-outs. Given a residual mine water concentration of 100 mg/L-U for every 100,000 litres of solution with 1% biomass you would get 11.7 kg of uranium (U_3O_8) in the water and 23.8 kg of uranium in the biomass. On surface the biomass would be eluted with bicarbonate or a strong acid solution, discarded and/or recycled and the soluble uranium sent to the mill for recovery.

The net effect would be:

- . mine waters would be recycled underground
- . effective uranium concentration in mine water pumped to surface is tripled. i.e. you get three times more uranium to surface in the same volume of mine water.

The major problem would be getting an adequate supply of biomass. For example a typical municipal sewage plant would produce .5 kg biomass per kg of BOD_5 applied. For a community of 20,000 people you could only produce 1,000 kg of biomass per day. This would only support the production of 23 kg of U_3O_8 per day. In addition the sludge would require thickening before pumping underground (10% solids would be reasonable). Using a

centrifuge or vacuum filter it should be possible to thicken and recycle the sludge. For a 10 cycle operation, this would increase the potential production to 230 kg/day or 84,000 kg/a. Total pumping capacity required at 1% solids to support this level of production would be 1,000,000 L/d. Obviously if the adsorption capacity could be improved, much greater levels of uranium could be produced.

3.3 Acid Tailings Runoff

Acid runoff and seepages from uranium tailings areas contains elevated levels of uranium however these levels are in the range of <1-5 mg-U/L with pH levels in the 2.2-3.5 range. These runoff and seepage streams require continuous long term treatment at a substantial cost. With the low uranium values in solution, conventional recovery technologies may be applicable however the costs would be prohibitive. In these situations, biomass could very well prove to be effective for uranium recovery. The uranium recovered could offset the cost of treatment and spent biomass could be used as a surface soil conditioner for the tailings. This would provide:

- . potential revenue from uranium
- . method for disposing sewage sludge
- . provide improved conditions for tailings area revegetation and rehabilitation.

For this application sludges could be added upstream of sedimentation pond and allowed to settle and contact with the seepage. Once or twice per year, the sludge would be recovered, eluted for its uranium content and the waste material disposed on the tailings surface.

3.4 Bioleaching Solutions

One of the potential applications is the recovery of uranium from heap leaching or underground leaching operations. These operations produce a uranium content typically in the range of 100 to 300 mg/L. For a heap leaching operation on surface, ion exchange or solvent extraction technology would likely be the simplest operation but underground recovery with biomass certainly has some potential. Again the driving force would be to have an inexpensive adsorber that does not require major process controls. The bioleach process solutions tested in Stage II of this study had a very high acid content, at pH of 2.0 and a uranium level of approximately 250 mg/L. At the low pH, the biomass was degraded. It will therefore be necessary to perform some pretreatment to adjust the pH upwards prior to adsorption. This pretreatment may also have some beneficial side effects as under the current scheme the leaching solutions are too strong and actually can inhibit bacterial activity.

For this type of application either a system similar to that discussed for the mine water would be employed or possibly a contacting system using columns could also be considered. Both of these applications are expanded upon in Section 4.0.

Again the benefits are

- . direct underground recycle of leach water
- . higher production levels for the same quantity of mine water pumped.



4.0 EVALUATION OF BIOMASS SOLIDS CONTACT/SEPARATION EQUIPMENT

4.1 General

There are two basic types of contacting equipment stirred reactors and columns. Both have potential application for uranium bioadsorption. Solids separation devices include thickeners, filters and centrifuges. Given the difficulty in dewatering many biomasses the thickener and centrifuge systems are probably most applicable, however, filters can and have been used. The centrifuge is probably most applicable when wet recycle of the biomass is required.

In order to demonstrate the potential requirements for contacting/separation for a uranium recovery system, two sample flowsheets have been developed. The basic design criteria with regard to loading capacity, elution requirement, etc. have been derived from the Stage II laboratory test work. The process solution is Denison leach water at 250 mg/L uranium and pH adjusted to 2.5-3.5 range.

This section also includes an overall assessment of a range of other contact/separation devices and how amenable these devices are for each of the biomasses tested.

4.2 Sample Biomass Recovery Flowsheets

4.2.1 Contacting System

The size of the equipment necessary to remove soluble uranium from a typical process mine water has been estimated for stirred tanks and gravity downflow column systems. The design parameters are considered reasonably conservative but require confirmation.

4.2.2 Design Criteria (from laboratory testwork)

Feed solution

flow	40 m ³ /h
pH	2.7
U concentration	0.250 kg/m ³
Fe concentration	0.27 kg/m ³

Discharge solution

flow	40 m ³ /h
U concentration	0.150 kg/m ³

U recovery	96 kg/d
------------	---------

Biomass

net U loading	0.02 kg/kg (dry)
percent solids, settled	3
percent solids, drained	10
percent solids, centrifuged	20
settling rate	0.8 m/h
bed percolation rate	0.6 m/h

Contact time

adsorption	30 minutes
elution	15 minutes

Eluant

sodium bicarbonate	8.4 kg/m ³
flow	2.0 m ³ /m ³ pulp



4.2.3 Stirred Tank System

i) Design

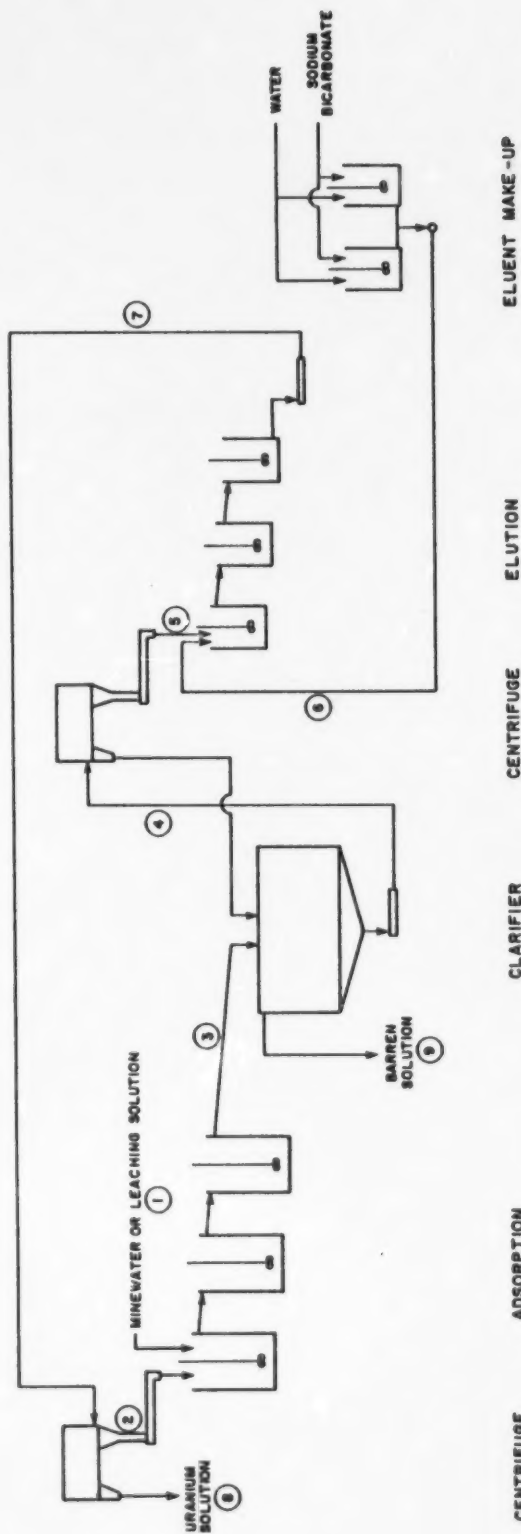
The flowchart is shown on Figure 4.1. Mine water and biomass are contacted in a series of three stirred tanks providing a total contact time of 30 minutes. Sufficient biomass is introduced to reduce the uranium content of the water by an average of 0.1 kg/m^3 .

The water/biomass pulp gravitates from the final tank to a gravity clarifier. The water overflowing at the top of the vessel and is recycled to a leaching stoep. The solids settle to the bottom of the clarifier and are raked to the centre to be pumped out as a 3% solids pulp. Typically such clarifiers are provided with a deep centre well and with a thick bed of suspended solids which act to filter the upflowing solution. Progressing cavity pumps are used on biomass pulps in order to avoid physical degradation of the material. Flocculants are generally not used during clarifications but could prove beneficial in improving underflow densities.

The thickened biomass is directed to a solid bowl centrifuge. The extracted water is returned to the clarifier and the biomass, now at 20% solids, is directed to the elution tanks. Uranium is eluted from the biomass with a 0.1 Molar (8.4 kg/m^3) solution of sodium bicarbonate. Fifteen minutes contact time is provided to transfer uranium from the biomass into the solution.

A minor amount of bicarbonate is destroyed by the acid

FIGURE 4-1
PRELIMINARY FLOWSHEET
FOR URANIUM RECOVERY FROM MINERWATER
USING BIOMASS AND STIRRED REACTORS



NOTE:
SEE SECTION 4.2.2. FOR EQUIPMENT SIZING

	1	2	3	4	5	6	7	8	9
SOLUTION FLOW (m^3/h)	40	1-0	41	8-08	1-0	4-0	8-0	4-0	40
BIOMASS FLOW (kg/h)	-	250	250	250	250	-	250	-	-
URANIUM CONCENTRATION (kg/m^3)	0-250	-	0-15	-	-	-	1-0	1-0	0-15
URANIUM CONCENTRATION NET (kg/kg)	-	-	0-020	0-020	0-020	-	-	-	-

in the water introduced with the biomass. If found advantageous to do so, this amount could be reduced by repulping the solids with clean water and separating in a second centrifuge.

Pulp overflowing the second elution vessel is pumped to another centrifuge. It would be located above the contactors so that a screw conveyor could be used to assist in returning the thickened biomass to the contactors. The centrate is directed to a mine sump and pumped to surface.

The amount of uranium returning to the contactor with recycled biomass could be reduced by just repulping the biomass with water and centrifuging the pulp in a third machine. The consumption of sodium bicarbonate would be reduced thereby.

ii)

Comments

1. The system appears simple, the equipment of familiar type and size, and the operation straightforward.
2. Replacement of biomass with fresh material is readily accomplished.
3. The sodium bicarbonate consumption is dependent upon a sufficient percent solids in the biomass discharge from centrifuges. The moisture in the material may vary and with the age and condition of the biomass.



iii) **Equipment List**

Contactator tanks	3	2.0 m dia. x 2.4 m, agitated
Clarifier	1	8 m dia.
Slurry Pump	2	Moyno type, variable feed drive
Centrifuge	2	420 mm dia. x 1675 mm, 37.5 kw
Elution tanks	3	1.0 m x 1.4 m, agitated
Bicarb. mix tank	2	4.0 m dia. x 4 m, agitated
Bicarb. feed pump	1	centrifical
Screw Conveyor	2	225 mm dia. x 3 m
Est. sodium bicarb.		11 kg/kg U
		1040 kg/d
est. connected power		105 kw

iv) **Capital Cost**

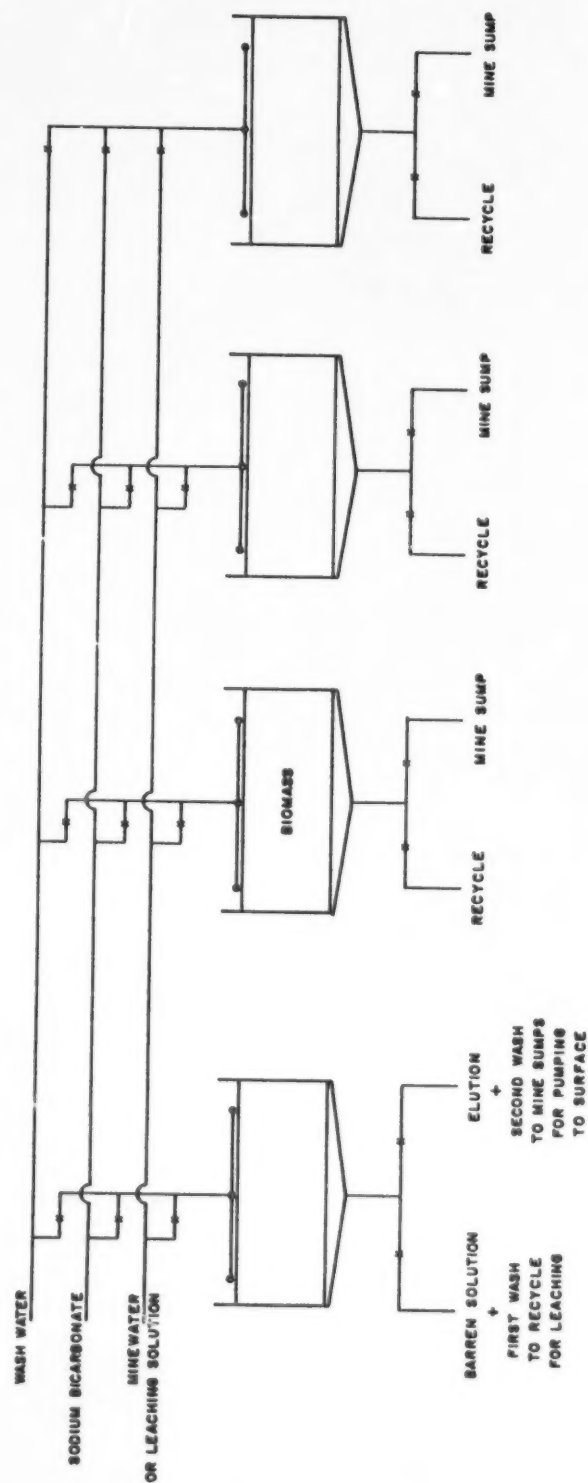
A rough estimate of the capital cost would be \$1,000,000.

4.2.4 Column System

i) **Design**

The flowsheet is shown in Figure 4.2. Mine water and biomass are contacted by passing the water under gravity head down through a bed of biomass. After the passage of a measured amount, the mine water is stopped and displaced from the bed with a flow of fresh water. The fresh water is followed by sodium bicarbonate elution and a second water wash. The water washes reduce contact between the acidic mine water and bicarbonate solution, avoiding disruption of the bed

FIGURE 4-2
PRELIMINARY FLOWSHEET
FOR URANIUM RECOVERY FROM MINEWATER
USING BIOMASS AND COLUMNS



NOTE:
SEE SECTION 4.2.3 FOR EQUIPMENT SIZING

that would be caused by the release of carbon dioxide.

The rate of water percolation through the bed is in the low and a large bed area is required to pass the design flow. Mine water would be sprinkled into a shallow pool on the top of the bed. Pressure on the bed is avoided in order not to compact the bed and thereby restrict the flow.

Treated mine water is collected with a bed support system at the bottom and recirculated to leaching areas. Outlet eluant is directed to mine sumps for pumping to surface.

A spare column to permit normal operation during replacement of a bed is provided.

ii)

Comments

The column equipment is simple and the system cheaper to install and operate than the stirred tank system. Operation should require little attention except for periodic replacement of the beds and routine make-up of the sodium bicarbonate eluant. The chances of successful technical operation, however, appear less certain. Potential problems with high turbidity resulting in column plugging are also possible.

Incomplete separation of mine water and eluant and the resulting CO₂ generation may disrupt the bed and lead to significant channelling of solutions flows. This would reduce adsorption/elution effectiveness and provide uranium recovery lower than expected.

The throughput is critically dependent on percolation into and through the bed. Stability of the flow through rate as a function of prior treatment of the biomass and operating conditions requires confirmation.

iii) **Equipment List**

<u>Operating Cycle</u> <u>Operation</u>	<u>Time</u> <u>Hours</u>	<u>Volume</u> <u>m³</u>	<u>Rate</u> <u>m³/h</u>
Adsorption	19.2	240	12.5
Wash	1.44	18	12.5
Elution	1.92	24	12.5
Wash	<u>1.44</u>	<u>18</u>	<u>12.5</u>
TOTAL	24	300	12.5

Wash water consumption	1.5 m ³ /kg
Sodium bicarbonate consumption	8.4 kg/kg U
	806 kg/day

Biomass bed, 5 @	12 m ³ , 5.2 m
Eluant make-up tanks, 2	dia. x 0.57 m
Eluant feed pump	agitated
	centrifugal

iv) **Capital Cost**

A rough estimate of the capital cost for this type application is \$700,000.

4.3 Overall Comparison of Contacting/Separation Devices

A summary of the expected performance of the various biomasses



with the principal solids contacting separation devices is provided in Table 4.1. A brief discussion of each is provided below.

ADSORPTION DEVICES

i) Columns

Column certainly have potential for usage. Primary concerns still exist over the structural changes of all biomasses under repeated adsorption/desorption cycles and how these changes will effect flow through characteristics. Given the unknowns, the mixed reactors would likely be more reliable. Rhizopus arrhizus had the best flow through characteristics and mechanical properties and for thin beds could possibly be utilized without immobilization. All other organisms require immobilization. Algae may also require agglomeration with sand.

ii) Mixed Reactors

The mixed reactor should be acceptable for all organisms assuming solids separation devices are available to provide adequate concentrations of biomass in the reactor.

SOLIDS SEPARATION DEVICES

i) Thickeners

Thickeners or clarifiers are commonly used for concentrating biomasses. Heat treated biomasses are more readily concentrated and dewatered than viable

TABLE 4.1

BIOMASS ADAPTABILITY TO CONVENTIONAL SOLID CONTACTING/SEPARATION

PROCESS EQUIPMENT		<u>Rhizopus</u> <u>arrhizus</u>	<u>Streptomyces</u> <u>leворis</u>	<u>Mixed</u> <u>Culture</u>	<u>Saccharomyces</u> <u>cerevisiae</u>	<u>Chlorella</u> <u>vulgaris</u>
ADSORPTION DEVICES						
. columns		direct use may be possible	immobilization is required	immobilization is required	immobilization is required.	not suitable without major treatment such as agglomeration with sand
. mixed reactor		no problem	no problem	no problem	no problem	no problem
RANK ³		1	2	2	2	3
Solids Separation Devices ²						
Thickeners		acceptable 2-4% solids in underflow	acceptable 1-4% solids in underflow	poor .5-1.5% solids in underflow	acceptable 1-4% in the underflow	unacceptable
Filters						
. belt filter		good	good	good	good	good
. vacuum drum filter		good 15-20% solids	good 10-20% solids	good 10-20% solids	good 10-20% solids	acceptable
. filter press		excellent >25% solids	excellent >20% solids	excellent >20% solids	excellent 720% solids	adequate
Centrifuge		excellent 20% solids	excellent 20% solids	excellent 20% solids	excellent 20% solids	excellent 20% solids
RANK		1	3	2	3	5

NOTES

1. n/a means not applicable or not acceptable
2. all biomasses are assumed to be heat treated (inactivated)
3. rank - No. 1 is best, No. 2 second best, etc.

cells. Underflow concentrations will vary greatly between biomasses ranging from 2 to 4 percent for the fungus to .5 to 1.5 for mixed culture to <.5% for the algae.

ii) **Filters**

Three filters have been considered, the belt filter, the vacuum drum filter and the filter press. All three types of filters have been extensively studied for mixed cultures sewage sludges. Typical performances range from very poor to excellent. Again, thermal inactivation greatly improves dewaterability.

The vacuum drum filter and the belt filter should produce cakes in the range of 15-20% solids content for the fungus, and 10-20% solids range for the mixed culture. The Streptomyces, which produces a polysaccharide slime, is expected to cause filtration problems and the algae would be most difficult to filter.

The filter press is expected to be acceptable for all biomasses but again it would perform the best on the fungi.

iii) **Centrifuge**

The centrifuge is a common apparatus for both dewatering and concentrating biomasses. The major problems would be obtaining a sufficient solids concentration in the inlet feed for biomasses such as the algae which would be difficult to preconcentrate in a thickener.

5.0 MAJOR ECONOMIC HURDLES

The primary economic hurdles are the biomass cost, capital cost of equipment and the operating cost for desorbing agents and labour. A short discussion of each is provided.

i) Biomass Cost

Although the two primary candidates Rhizopus arrhizus and the mixed culture are either available as waste by-products or can be produced from waste materials, transportation, treatment, dewatering, etc. will still contribute greatly to the cost. A major factor will also be the scale of production. If the demand is small the cost will be very high. Conversely, if demand is great, the economy of scale will play a major role in reducing costs. Realistically a cost of \$3 to 5/kg would be appropriate to permit culturing, inactivation and transportation. For immobilization an additional 1.00/kg should be added.

From this assessment, we consider 10 cycles the best that could be expected for reusing the biomass. For this use level and a net effective 20 mg/g biomass adsorption capacity, a total of 4.3 Kg of biomass would be required per Kg of U_3O_8 . At \$4/Kg for biomass and \$50/kg for U_3O_8 the biomass represents 34% of the cost of the uranium product or approximately \$17/kg of U_3O_8 .

ii) Capital Cost of Equipment

A very preliminary estimate for the capital cost of equipment for the underground recovery of uranium has been undertaken using the stirred reactor type adsorption flowsheet. Approximate costs for this facility would be \$1,000,000. Assuming a 3-year amortization at 12 percent, borrowing and capital payback cost represents approximately \$9.65/kg of U_3O_8 .

iii) Elution Agent

Sodium bicarbonate is the most effective agent and approximately 9 Kg bicarbonate are required per Kg of U_3O_8 . At 40 cents/Kg for bicarbonate this represents approximately \$3.60/Kg U_3O_8 .

iv) Labour

The labour requirement would likely be 1 full-time operator/labourer, 2 shifts/day. At \$50,000/a labour cost per person this would represent approximately \$2.43/Kg U_3O_8 .

v) Miscellaneous

Ongoing maintenance, power cost would be added to the above costs but are not expected to add substantially to the production costs.

From the above discussion it can be seen that biomass and capital costs are the most significant factors. Potential cost could be in the range of \$30-35/kg of U_3O_8 .

6.0 MAJOR TECHNICAL PROBLEMS

The major technical problems which would effect the full scale application of biomass for uranium recovery are expected to be:

- . production of an immobilized culture at a reasonable cost which has good bioadsorption characteristics in combination with mechanical strength.
- . maintenance of the bioadsorption characteristics and mechanical properties in cyclic adsorption/desorption operations.
- . small scale production of biomass. With limited demand, costs for biomass could be very prohibitive.
- . Cost competitive applications as compared to conventional technologies.
- . for underground applications, concerns over handling chemicals, and CO₂ generation from bicarbonate.
- . contamination and degradation of the biomass by facultative and anaerobic bacteria. Development of septic conditions and odours.
- . Instrumentation of the uranium recovery system for optimum performance.

7.0 OVERALL COMPARISON AND RANKING OF BIOMASS

A summary of the major features for each of the biomasses is provided in Table 7.1. A brief discussion of the major features is given below.

i) Adsorption

The ability of any microorganism to adsorb uranium is a function of numerous parameters. These include uranium concentration, contact time, microorganism surface area, pH, competing ions etc. Conditions can be created where any of the organisms tested will appear to perform better. As a general rule the bioadsorption capacity of all organisms is similar and no one organism is greatly better than the others. Rate of adsorption was also highly variable and was most likely attributable to the relative surface area of organisms available for adsorption.

None of the organisms was selective to uranium. In this regard both cations (Fe^{+3} , Ni^{2+} ...) and anions (SO_4^{2-}) interfere with adsorption capabilities.

ii) Cultivating

The microorganisms excepting the alga were simple to cultivate and harvest. Growth rate and yields for the organisms are similar. The Rhizopus arrhizus, Saccharomyces cerevisiae and the mixed cultures require simple media which is likely available as waste material at no cost. This a major consideration as biomass purchase costs are very high.

None of the organisms was readily immobilized and this is a major problem where column type applications are considered.

Table 7.1

OVERALL COMPARISON OF BIOMASS CHARACTERISTICS

	<u>Rhizopus arrhizus</u>	<u>Streptomyces levoris</u>	<u>Mixed Culture</u>	<u>Saccharomyces cerevisiae</u>	<u>Chlorella vulgaris</u>
ADSORPTION ASPECTS					
Equilibrium Capacity pH=3.5, u=243 mg/L process solution	42.3	62.3	49.9	50.8	28.5
Relative rate of Adsorption (k) 20°C, pH 3.5	3.27	4.23	10	5.82	-
Selectivity for uranium	poor	poor	poor	poor	poor
CULTURING ASPECTS					
Growth Rate hr ⁻¹ Media Req't	0.2 simple -potato waste -trace nutrients	0.22 complex -sugar & tryptone -trace nutrients	n/a simple -any biodegradable organic waste -trace nutrients	0.24 simple sugar, yeast extract tract nutrients	.033 complex -glucose, yeast extract, buffers -multiple trace nutrients
Typical yield g/g Cost	0.46 moderate	0.32 high	n/a moderate	0.42 high	0.12 very high
Contamination potential	low	moderate	low	moderate	high
Ease of Immobilization	all of the organisms can be immobilized. However, the success when exposed to process solutions remains to be demonstrated. Costs are prohibitive.				
DESORPTION agents 0.1M NaHCO ₃	>80% readily achieved	>90% readily achieved	>90% readily achieved	80% readily achieved	>90% readily achieved
REPEATED CYCLES					
Adsorption Capacity	reductions with each cycle - typically in the range of 10% reduction per cycle (i.e. after 10 cycles, the capacity is reduced about 40% of the capacity in the first cycle.				
Desorption Efficiency	generally unaffected for all biomasses tested				
Physical Aspects	Microorganisms tested can withstand repeated cycles at pH 3.5 and possibly into the 2.5 range. Cell degradation occurs at pH 2.0. Overall, Rhizopus performed superiorly without immobilization and mixed culture was superior with immobilization				
Mechanical Properties	good	moderate	moderate	moderate	poor
OVERALL RANKING	1	3	2	3	5
Overall Promise for Application	good	moderate	good	moderate	poor

iii) Desorbtion

Bicarbonate solutions were the only effective desorbtion agents. Typical recoveries are in the range of 90%. This is of little concern as wasted biomasses could be leached to extract virtually all of their residual uranium.

iv) Repeated Cycles

Work undertaken on repeated cycles of adsorbtion/desorbtion have shown that the organisms tested perform similarly. Over a 10 cycle operation, adsorption efficiency from the first to the tenth cycle can be expected to drop to 40 percent (i.e. an average of 70%). Desorbtion efficiency was not greatly effected. Mechanically, the Rhizopus was superior.

v) Overall Ranking

1. Rhizopus arrhizus (or other Rhizopus species) is ranked No. 1 for the biomasses listed. The prime reasons are the good mechanical properties, relative ease in settling/dewatering and the comparatively low cost of purchase.
2. Mixed culture is ranked No. 2 primarily for its low cost, ease of supply and generally overall good performance.
3. Streptomyces levoris is ranked No. 3. This organism is equally as effective as the mixed culture or Rhizopus arrhizus, however the higher purchase costs make it less attractive.
4. Saccharomyces cerevisiae is also ranked No. 3. The

major disadvantage of this organism is its poor mechanical properties and poor dewatering characteristics in acidic solutions. Purchase cost, however, is comparatively attractive.

5. Chlorella vulgaris is ranked No. 5. The algae has poor mechanical properties, is difficult to dewater, and is very expensive to culture heterotrophically in a pure state. Under autotrophic conditions, the specific growth rate is very low.

Based upon the foregoing we would suggest that Rhizopus arrhizus and the mixed culture show the great potential for application followed closely by Streptomyces levoris, and Saccharomyces cerevisiae. Chlorella vulgaris is not likely to be competitive for a full scale application.

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